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LEPTOMONAS SERPENS N.SP., PARASITIC IN THE DIGESTIVE TRACT AND SALIVARY GLANDS OF *NEZARA VIRIDULA* (PENTATOMIDAE) AND IN THE SAP OF *SOLANUM LYCOPERSICUM* (TOMATO) AND OTHER PLANTS

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(With 1 Figure in the Text)

A leptomonad parasite was found in the sap of the tomato and in one unidentified plant, but it has not yet been seen in several others on which the insect feeds.

Interesting characteristics of this *Leptomonas* are (*a*) its extreme polymorphism, (*b*) the development of metacyclic forms in the salivary glands of its insect host, and (*c*) its alternation between insect and plant host.

DEVELOPMENT IN THE INSECT HOST

The *Leptomonas* is found in the hind-gut and salivary glands of *Nezara viridula*, a sap-feeding bug found on a variety of wild and cultivated plants at the Cape Peninsula, South Africa. The insect, which is also found in Australia and in the East, possesses an extremely fine, needle-like proboscis by means of which it punctures the fruit and stems of certain plants and withdraws sap. The eggs are laid on the underside of the leaves and hatch as nymphs which, after remaining clustered together for 2 or 3 days, separate and become sap-feeders. During the present investigation the insects were gathered from the fruit of tomato plants, to which they are very partial.

Gut forms

In the digestive tract of the insect the parasites are confined almost entirely to the hind-gut; only rarely have a few leptomonads been found in the mid-gut. During the earliest observed phase in the experimental infection of a fresh host a few aflagellate forms measuring about 7 μ long (Fig. 1*a*), together with thin, twisted leptomonads about 10 μ long possessing flagella of about one body length (Fig. 1*b*) are found in the hind-gut. The location of the nucleus is not constant; it generally lies within the anterior half of the body, but individuals are found in which the nucleus is centrally situated. The kinetoplast lies near the anterior extremity.

When the gut becomes heavily parasitized the typical leptomonad is long, tenuous and very twisted. Its average length is about 40 μ and its width about 1·5 μ. The posterior third of the body tapers gradually and ends in a fine filament while the anterior end tapers more abruptly. Characteristically the nucleus is located within the anterior third of the body, the kinetoplast being set well forward (Fig. 1*c*). The flagellum measures about one-half to three-quarters of the

body length and moves in a remarkable serpent-like manner, usually forming three or four undulations which move continuously towards the body. Near the body the undulations have a depth of about two or three times the maximum body width, becoming more shallow nearer the tip, which does not oscillate from side to side, or take any part in the undulating movement, but merely glides along in an anterior direction. These characteristics of the flagellum can best be studied when the leptomonad has been allowed to become somewhat less active. Apparently the undulations are always on the same plane relative to each other, as when the organism is suspended in a liquid medium it is possible to focus the entire flagellum at the same time if the undulations are on the same plane as the microscope stage. If, on the other hand, the flagellum is moving in a direction vertical to the stage it will be possible, under suitable focusing, to view merely the apexes of the individual undulations moving in a posterior direction. The body is supple, the alternating lateral thrust of the flagellum causing slight bending of the anterior third, the remaining two-thirds being unaffected.

There are usually also present a number of short, stumpy leptomonads with flagella of varying lengths, but, except in the case of those individuals with very short flagella, the serpentine action of this organelle is constant. The powers of locomotion of all forms of the organism are feeble; in a liquid medium such as normal saline they are able to make head-way, but only in a slow and almost gregarine-like manner. They progress better through a semi-solid medium such as the gut contents.

On very rare occasions abnormally large leptomonads measuring as much as 100μ long have been found in the gut. In these cases the body is twisted while the posterior tip remains drawn out into a fine filament; the cytoplasm, however, appears coarse and granular during life and may even be in a state of brownian movement while the flagellum remains feebly active. Like the more typical leptomonad the nucleus is located within the anterior third of the body. The flagellum is short and ineffective.

No development takes place in the rectum, and although a few leptomonads may be found in the faeces no forms have been discovered which could be regarded as possible cysts.

Encapsulation in gut

There is no true leishmania-like stage in the life-history of the *Leptomonas*, a few rounded forms may occasionally be found in the gut, but these result from the phenomenon of 'encapsulation'. In a paper (Gibbs, 1951) describing *Leptomonas capsularis* in *Cletus ochraceus*, another sap-feeding insect, a description was given of the encapsulation of that organism. The present *Leptomonas* occasionally exhibits an almost identical phenomenon. First, the posterior half of the body becomes folded against the forward half. The parasite periodically endeavours to straighten but always reverts to its previous folded state. Living encapsulating individuals of *Leptomonas capsularis* at this stage exhibit a clearly visible film of transparent, gelatinous and exceedingly elastic substance, probably produced by secretion, which is particularly obvious in the angle formed between the two limbs

of the body during its efforts to straighten. In the present organism this substance is not so obvious at the same early stage of encapsulation, although undoubtedly it exists and can be seen. Later, the body remains folded, while the flagellum continues to be free and active. As encapsulation progresses the body becomes ring-shaped and continues in a state of vigorous wriggling movement, the hollow centre being occupied by the gelatinous material within which the flagellum is periodically retracted. When extended, the flagellum exhibits the usual serpentine action. Later, the entire organism, including the flagellum, becomes invested in a gelatinous mass which conforms to its every change of shape and forms an elastic, enveloping sheath to the flagellum when it is protruded (Fig. 1*d*). This stage is followed by a period during which the flagellum is no longer protruded, but may be observed to move vigorously within the central area of the capsule. Later the organism becomes quiescent and may present a leishmania-like appearance when dry-fixed and stained, although the flagellum can usually be discerned. The capsule is not visible in stained preparations. Encapsulated forms are incapable of locomotion.

The subsequent development of encapsulated forms has not been followed with any certainty but the occurrence of one or two divisions within the capsule is usual, after which it is likely that the enveloping material is either absorbed or dissolved and the resultant individuals separate.

Forms in salivary glands

At an early stage of the parasitization of the gut, and while the leptomonads are yet comparatively sparse, the salivary glands become involved. The path followed by them in their migration from the gut is not known, but it is presumed that the flagellate moves forward until it reaches the salivary channels. (It is of interest to note that the gut and salivary glands of *Nezara* are very frequently heavily infected by a yeast. The incidence of infection by this fungus is actually higher than that by the leptomonad. Like the leptomonad it becomes established in the gut before appearing in the salivary glands. No suggestion is offered here as to the means by which these non-motile cells gain entrance to the salivary glands.)

A constant characteristic of the leptomonads found in the salivary glands, with the exception of certain giant forms to be described, is the delicate manner in which they become coloured by Leishman's or Giemsa's stain. Often it is almost impossible to stain the cytoplasm, the chromatic bodies alone revealing the presence of the parasite. In the living state also they are difficult to discern and, when lying in the liquid contents of the gland, may be practically invisible owing to refractive similarity. They are best studied in saline.

The first leptomonads found in the salivary glands averaged some 25μ in length (Fig. 1*e*). The flagella may measure up to one body length. Only those with the longer flagella are capable of even very feeble travel. The flagella exhibit the same deliberate, serpentine movement described for the gut leptomonads. Later, longer forms appear which possess only very short flagella, the anterior end of the body being broad while the posterior two-thirds is extremely attenuated. The nucleus remains anteriorly situated. There is movement of the flagella but these organisms

are quite incapable of travel: it seems likely that the flagella act as anchorage within the salivary glands rather than as a means of locomotion.

At a later stage, smaller leptomonads, with very short and feebly-moving flagella, are also produced, and then small aflagellate forms appear in large numbers. These measure about 7 μ in length and have a maximum width of about

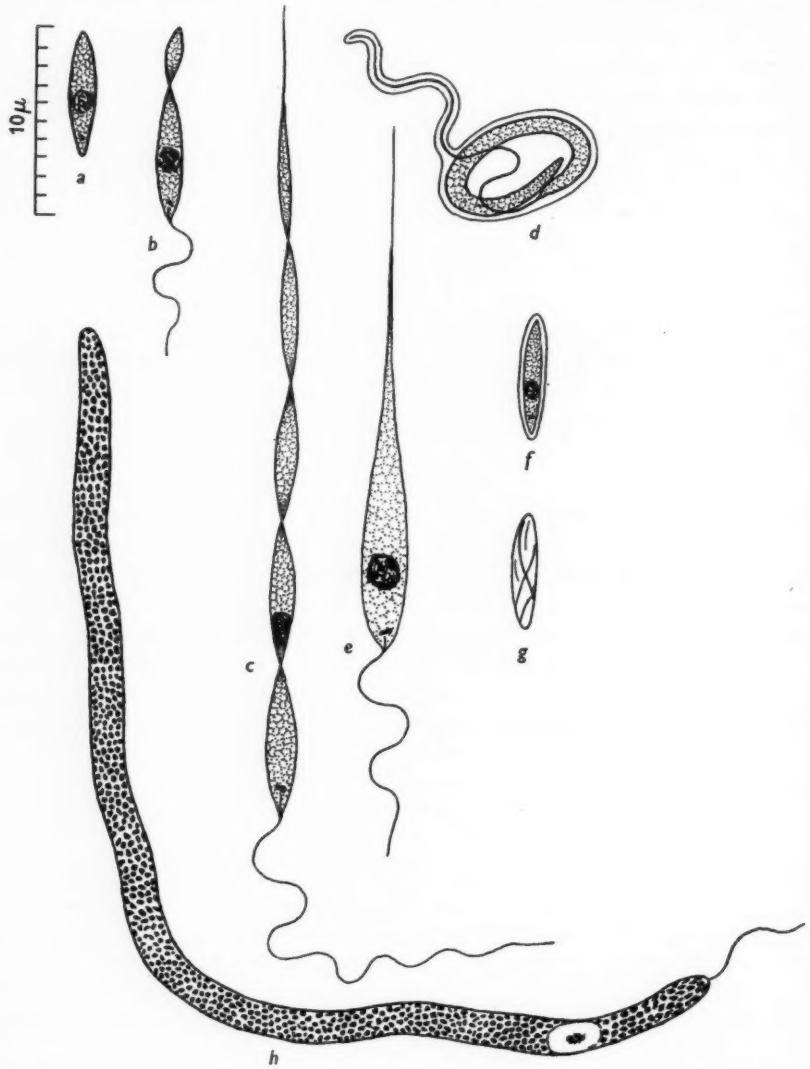

Fig. 1. *Leptomonas serpens* n.sp. *a*, *b*, First forms in the gut of experimentally infected nymph; *c*, typical leptomonad in gut; *d*, encapsulating leptomonad from gut; *e*, salivary gland leptomonad; *f*, small aflagellate form in salivary gland showing development of protective pellicle; *g*, metacyclic form; *h*, giant leptomonad from salivary gland. *Note*: *d*, *g* and *h* were drawn from the living organism, all other figures were drawn from material stained by Leishman's method.

2·2μ. From these forms small bodies are then produced by division which develop directly into metacyclic (infective) bodies of the same dimensions. An envelope of transparent material about 0·5μ in thickness is secreted around the latter (Fig. 1*f*); this afterwards contracts into an apparently tough, protective pellicle which, in the living state, exhibits ridges running diagonally across the body (Fig. 1*g*), but in stained preparations the ridged pellicle is not easily demonstrated. The contained organism, however, showing a distinct nucleus and kinetoplast, is clearly revealed.

When wet-fixed and stained with Ehrlich's haematoxylin and eosin, the nucleus in all flagellated forms of the *Leptomonas* is vesicular and exhibits a very heavy central karyosome, but in the metacyclic form the nucleus may consist of two closely associated parallel rods of chromatin lying along the long axis of the organism, or it may appear as an almost solid block of chromatin.

When the glands are heavily infected, it is not unusual to find a number of enormously over-grown forms; the largest of these usually have almost inconspicuous or no flagella and may measure over 150μ in length. When studied alive the cytoplasm in these forms appears dark and granular, while the nucleus, which is situated within the anterior fifth of the body, is clearly visible during life and exhibits a distinct karyosome. These larger parasites are often almost cylindrical in form (Fig. 1*h*), but twisted individuals with pointed posterior tips are also found. When stained by Leishman's method the cytoplasm is deeply coloured, although in a patchy manner. Both the nucleus and the kinetoplast may occasionally be masked by the opacity of the cytoplasm in stained preparations. Every gradation exists between these giants and the smaller, delicately staining leptomonads previously described.

DEVELOPMENT IN THE PLANT HOST

During the act of feeding, the sucking apparatus of the adult insect is inserted to a depth of about 6 mm. The insects do not move around making numerous punctures in the fruit but rather continue to feed from one spot for a considerable time if undisturbed. In captivity they have been observed to feed like this for several hours. Saliva is injected into the puncture as feeding proceeds and, if the salivary glands are heavily infected with *Leptomonas serpens*, metacyclic bodies are inoculated into the sap in large numbers. They can readily be demonstrated if sap is withdrawn from the tomato in the vicinity of the puncture. Within 24 hr. of being inoculated into the fruit the metacyclic bodies lose the ridged, protective pellicle, grow flagella and commence multiplication. The leptomonad has been found in active condition in the fruit 6 weeks after the last occasion on which it had been fed upon by infected insects. The plant has, therefore, to be regarded as an alternate host and not merely as a reservoir of inert, infective forms.

The average leptomonad in the sap measures 12μ long and possesses a flagellum of about one-half the body length, which does not exhibit quite the same serpentine movement as described for the organism while in the insect, its action being more vibratile in nature. Many of the leptomonads have even smaller flagella, and in others this organelle is entirely missing. The smallest aflagellate forms resemble the

metacyclic bodies exuded with the saliva of the insect. As such forms have been found in the sap 6 weeks after all further sources of infection have been withdrawn, two possible conclusions may be drawn: either (*a*) certain of the metacyclic bodies remain invested in their tough pellicle and do not take on leptomonad form, or (*b*) metacyclic bodies are actually produced in the sap in a manner similar to that occurring in the salivary glands.

Even the most active leptomonad in the sap is capable of only a very feeble rate of travel. Giant forms have never been observed in the plant.

Experimental infection

The infection of clean-bread nymphs was achieved by allowing them to feed upon tomatoes which had been heavily infected by metacyclic forms of the *Leptomonas*. Insects were gathered from a locality where it was known that the incidence of the *Leptomonas* among adults was about 90 % and, in captivity, these were allowed to feed upon young, green, picked tomatoes for 3 days. The tomatoes were then washed and set aside for 10 days. Ten nymphs were then gathered from an isolated area where infection by the leptomonad had never been observed. These were allowed to feed, in captivity, on the infected tomatoes, while another ten nymphs, gathered from the same locality, were allowed to feed on uninfected tomatoes. After 7 days all of those which had fed upon the infected tomatoes were found to harbour the parasite in the hind-gut while all controls were completely negative.

DISCUSSION

The life cycle of this *Leptomonas* alternates between the pentatomid bug *Nezara viridula* and several plants (especially tomato) at the Cape Peninsula. The parasite is inoculated into the plant by the insect during the act of feeding, and a new insect becomes infected by feeding on the infected plant. Although a few leptomonads may be found in the faeces of the insect, nothing has been discovered which might suggest that transmission is possible by faecal contamination.

On very rare occasions (in less than 1 % of the insects studied) an infection by *Crithidia familiaris* (Gibbs, 1950), which is commonly found in the sap-feeder *Cenaeus carnifex* at the Cape Peninsula, has been observed in the gut of *Nezara viridula*. It is interesting to note that when the insect becomes infected by *Crithidia familiaris* the salivary glands do not become involved, as this organism produces infective, resistant bodies in the gut which are disseminated with the dejecta.

In an unpublished thesis entitled 'The relation of the milkweed bug, *Oncopeltus fasciatus* (Dall.), to flagellosis in laticiferous plants', Barnett (1946) described small herpetomonad (leptomonad) forms measuring 9·4 by 2 μ in the salivary glands of the insect, but did not find specialized resistant forms in that organ. He did, however, find encysted forms in the faecal deposits. He considers that, in the insect, the infection usually results from the ingestion of infected latex, but does not rule out the possibility of infection being brought about as a result of faecal contamination.

It is possible that the metacyclic (resistant) forms of our *Leptomonas* can pass directly from insect to insect without the necessity of a developmental phase in plant sap. In captivity the insects have been observed to exude from the sucking apparatus a droplet of saliva, which is then wiped off on one of the front legs, after which the legs are rubbed together, much in the manner of a house-fly. It is highly probable, therefore, that metacyclic bodies can be deposited on the surface of the fruit and may subsequently contaminate the proboscis of another insect. Since the metacyclic bodies are invested in a protective pellicle it seems possible that they may be able to withstand external exposure for a considerable time.

The described organism obviously qualifies for inclusion in the genus *Leptomonas*, and as it has not apparently been hitherto described the specific name *serpens* is proposed.

DEFINITION OF NEW SPECIES

Leptomonas serpens n.sp. is parasitic in the gut and salivary glands of *Nezara viridula* (fam. Pentatomidae) and in the sap of cultivated and wild plants at the Cape Peninsula, South Africa. The typical leptomonad found in the gut measures about 45μ in length and is tenuous and twisted. The nucleus is characteristically located within the anterior third of the body while the kinetoplast is set well forward. The flagellum measures about one-half to three-quarters the body length; its movement being serpentine in character. The organism is almost incapable of travel in a completely liquid medium, although it can make headway through semi-solid matter. Encapsulation is not an uncommon phenomenon in the gut.

In the salivary glands of *Nezara* the posterior portion of the body of the leptomonad is extremely attenuated. It averages 55μ in length and usually possesses a very short flagellum and is incapable of travel. Giant forms measuring as much as 150μ long are not uncommon.

The final product in the salivary gland is the metacyclic body which is about 7μ long. These forms have a ridged, protective ectoplasmic pellicle and are inoculated into a plant during feeding, after which many of them soon take on leptomonad form.

The average leptomonad found in the plant sap measures about 12μ long and has a flagellum equalling about one-half of the body length. Forms similar to the metacyclic bodies are also found in the sap.

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NEMATODE PARASITE POPULATIONS IN SHEEP ON LOWLAND FARMS

III. THE SEASONAL INCIDENCE OF SPECIES

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(With 9 Figures in the Text)

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I. INTRODUCTION

Various authors have recorded the seasonal occurrence of different species of nematode parasites in sheep. Some of these (Ross & Gordon, 1936; Gordon, 1950; Taylor, 1934) have been mainly interested in the epidemiology of outbreaks of parasitic disease, but others have recorded the incidence of different species in flocks in which no outbreak has occurred (Gordon, 1948, 1950; Morgan & Oldham, 1934; Morgan & Sloan, 1947; Morgan, Parnell & Rayski, 1950; Parnell, 1954; Parnell, Rayski, Dunn & Mackenzie, 1954; Stewart & Douglas, 1938; Tetley, 1935, 1941, 1949; Wilson, Morgan, Parnell & Rayski, 1953).

In a previous paper (Crofton, 1955) a brief statement of the times of occurrence of different species in south-west England was given. Further work has provided additional information and this, together with a general theory to explain species-succession, is presented here.

II. MATERIAL AND METHODS

Observations have been made on commercial flocks in south-west England since 1950. Samples of faeces have been taken at weekly or fortnightly intervals from lambs and ewes in these flocks. Each sheep was tattooed with a code number and could be identified individually as well as by flock. The techniques for worm egg counts and for differential egg and larval counts have been described previously (Crofton, 1954; Cunliffe & Crofton, 1953). Attempts have been made to correlate these counts with pasture infestation, but although a gross numerical relationship has been apparent, the difficulties of pasture sampling, which have been discussed previously (Crofton, 1952), have prevented any reliable estimates of individual species being made. From time to time post-mortem examinations have been made, but as these have been used mainly as tests of efficiency of other techniques, the details of worm counts will not be presented here.

III. RESULTS

The times of maximum numbers of different species in lambs were found to be as described previously (Crofton, 1955). The more detailed observations have shown that when the mean number of any particular species of parasite per host was low then the frequency of occurrence of infected hosts was low also. With increase in the mean number per host there was an increase in frequency of occurrence of infected individuals. When the flock was considered as a whole, all species of nematode parasites occurring in that flock were represented at all times of the year. It must be admitted that on some occasions the only evidence of the presence of a particular species was a low infection in one individual in the flock.

In ewes the succession of species was not marked, and seasonal variation in species constitution was not very apparent. In earlier observations this was thought to be the result of using relatively crude techniques for differentiation of species in samples with low egg counts. The present work has shown that although some variation occurs, most species are present throughout the year.

Such variations as occur appear to be closely related to the past history of the individual sheep, and no marked seasonal changes can be seen. This does not imply that the worm burden of ewes is static. The rate of change of parasite population in ewes appears to be much lower than in young sheep, and the relatively small numbers of worms which established themselves under conditions of high infection pressures are not sufficient to show any regular or marked species succession.

There are two possible exceptions to this. First, at the time of the post-parturient or Spring Rise there may be a considerable increase in the number of species which develop at low temperatures if the lambing is early. Later lambings are less likely to show a marked differential increase. Secondly, in the case of *Nematodirus* spp. there appears to be a marked season lasting from May to the end of August.

More detailed study of the post-parturient (or Spring Rise) has shown that while *Ostertagia* spp. made the greatest contribution to the rise in most ewes, all other species normal to the flock occurred in the flock as a whole, even though only one or two species might be found in many individuals.

In lambs the differences in incidence of different species was well marked. At no time was there a 'pure' infection of any one species, but at different periods of the year different species were more numerous than others. At any particular time one species could be regarded as dominant numerically. With minor variations the order of succession of dominant species was the same from year to year and from farm to farm in the same area. The actual numbers varied not only from year to year but also from farm to farm.

Details of the occurrence of different species in lambs are given below. The account is similar to that given previously (Crofton, 1955), but additional information has been obtained. It will be noted that the observations are in fair agreement with those described for Scotland (see Parnell *et al.* 1954).

Haemonchus contortus

Only small numbers of this species occurred, and in no case were the numbers of pathological significance. Low infections were detected in May and early June, and the numbers increased during the summer months. The heaviest infections occurred from July onwards, but even in this period the numbers were insufficient for any marked peak to occur.

The area considered does not fall within the limits suggested by Dinaburg (1944 *a*, *b*) and Gordon (1948, 1950); in only very exceptional seasons would the mean maximum temperatures reach 64–65° F. and the mean monthly rainfall approach 210 points. Thus the very low numbers of this species recorded are not surprising.

Ostertagia spp.

These were mainly *O. circumcincta*, but some *O. trifurcata* and *O. oestertagi* have been observed occasionally at post-mortem examinations. With the exception of *Nematodirus* spp., *O. circumcincta* was the species detected earliest in the year and it was present in appreciable numbers from early June onwards. The numbers increased rapidly and this species made the major contribution to egg-counts during spring and early summer. It was present in considerable numbers during August and September, but by these months other species had increased in number and it had ceased to be the 'dominant' species.

Trichostrongylus spp.

T. axei and *T. vitrinus* were the two most important species of this genus. *T. colubriformis* was detected when worm-counts were made, but it was a relatively unimportant species.

Small numbers of *Trichostrongylus* eggs occurred sporadically during early summer, but it was not until late June and July that appreciable numbers were detected regularly. The numbers increased during August and September. After the fall in total egg-counts during autumn (see Crofton, 1955) the numbers of these species were greatly reduced. In a few exceptional cases examined where the lambs did not exhibit spontaneous reduction in egg-count the numbers of *Trichostrongylus* increased until late in the year. It is significant that when cases of winter helminthiasis were examined the most numerous species were either of the genus *Trichostrongylus* or the genus *Cooperia*.

Cooperia curticei

C. oncophora was encountered on only rare occasions and in small numbers at post-mortem examinations and the bulk of *Cooperia* infections were almost entirely *C. curticei*. The numbers of this species were relatively low and, although small numbers were present sporadically from July onwards it was not until the main peak of egg counts in autumn that numbers were appreciable. This species was never as numerous as *Trichostrongylus* spp., although in the later part of the year (December–January) the number of *Cooperia* spp. made a considerable con-

tribution to the total number of worms in a hogg suffering from winter helminthiasis. In most lambs, however, the spontaneous fall in egg-counts occurred before large numbers of *Cooperia* spp. became established.

Nematodirus spp.

These were mainly *N. filicollis*, although *N. spathiger* was detected in small numbers in the later observations. During 1954–5 *N. battus* was recorded on one occasion. The relative numbers of these species were such that all *Nematodirus* records can be considered as *N. filicollis*.

This species was remarkable in that it had a restricted season in ewes and that the peak infections in ewes and lambs occurred about the same time. In each year the infection was first observed in April or early May, and the greatest numbers occurred in June. After July the numbers of *Nematodirus* eggs were small and eggs were found only occasionally during the rest of the year.

Bunostomum trigonocephalum

Eggs of this species were not detected until August and then only in small numbers and in only some samples. They occurred sporadically throughout autumn and winter but the numbers remained low.

Chabertia ovina

Only very small numbers of this species were detected in lambs and hoggs. Occasional high egg-counts recorded from August onwards occurred in a small number of samples, but these could be attributed to the inclusion of adult female worms in the faecal sample. There was a slight increase in numbers of *Chabertia* in December and January worm counts, but the numbers were still small.

Oesophagostomum venulosum

This species was not recorded until August. The numbers were slightly greater in December, but only very low-grade infections occurred.

IV. A THEORY OF SEASONAL INCIDENCE

Consideration of the incidence of the different species in lambs has led to the development of a theory to explain the phenomenon of a regular succession of 'dominant' species throughout the season. This succession has not only been fairly constant from year to year in the present work but, as has been remarked in a previous paper (Crofton, 1955), has shown the same serial order of succession in other areas.

It is suggested that the pattern of seasonal incidence is not necessarily one which can be attributed to seasonal changes of climate, but is a consequence of the life-history pattern of individual species, and is directly connected with the rate of egg production and the time required for development of each generation. (It is to be noted that Cole (1954) discussed some of the general consequences of life-history phenomena, and his excellent treatment of this subject has stimulated and to some extent justified the present approach.)

It has been shown previously (Crofton, 1955) that the increase of populations in lambs is of the logarithmic type so that each increment is proportional to the population existing immediately before, and that the rate of egg production is proportional to the intrinsic rate of increase of the population under these conditions.

We can express this as

$$P_t = P_0 E^t, \tag{1}$$

or if there is no mortality

$$P_t = P_0(1+E)^t, \tag{2}$$

where P_t is the number of individuals at time t, P_0 is the number of individuals initially, E is the intrinsic rate of increase of individuals and t is the time measured in generation intervals.

This is, of course, the general form applicable to a species increasing in an unlimited environment, and its use at this stage is justified in so far as initially it is only necessary to establish maximum rates of increase.

The question as to whether (1) or (2) above would be more applicable would appear to be of some importance parasitologically, but the form chosen is likely to have little effect upon the final result because the parameter E is to be estimated, and the question of semelparity or iteroparity need not be resolved (see Cole, 1954). It is proposed to use (2) because it would be convenient to assume that no mortality occurred over the short initial period and that later there was some mortality of old infections when (1) would then apply. This for the same value of E would describe the upper and lower limits of the intrinsic population increase corresponding to semelparity and iteroparity.

To calculate $P_t(1+E)^t$ we can chose a time such that $P_t = 1$ because in any season we can assume that lambs are born worm-free. From observation of flocks in this area we can assume that initial infections occur about 1 April. This may vary from place to place, but it is to be noted that any slight inaccuracy in choice of a starting-point can be neglected, particularly if the inaccuracy is less than the interval between generations.

For each species the time corresponding to a unit $t = 1$ can be evaluated from published data on the life histories observed in the laboratory. The minimum time between generations must be the sum of the time required for eggs to reach the infective stage and the length of the prepatent period.

In most species the prepatent period is usually regarded as constant but there are exceptions. In the species considered the only case in which great variation appears to be possible is that of *Oesophagostomum venulosum*; even in this case the amount of variation is likely to be considerably less than in the nodule-forming species *O. columbianum* which does not occur in this country.

The time taken for development from egg to infective stage is known to vary according to climatic conditions. We can, however, use the minimum time for development and study the effect of increasing this over-all possible variation.

The figures used for estimating the generation times are given in Table 1.

E cannot be determined directly but can be evaluated by choosing a species in which the seasonal incidence is well marked. Obviously the greatest sensitivity is

Table 1. *The data used for calculation of minimum generation time*

Species	Time in days: Free-living development	Prepatent period (I)	Generation interval
Haemonchus contortus	7	20	27
Ostertagia spp.	5	17	22
Trichostrongylus spp.	5	17	22
Cooperia spp.	5	15	20
Nematodirus spp.	14	28	42
Oesophagostomum venulosum	7	42*	49
Chabertia ovina	7	63	70
Bunostomum	7	70	77

* An arbitrary figure chosen for *Oesophagostomum*.

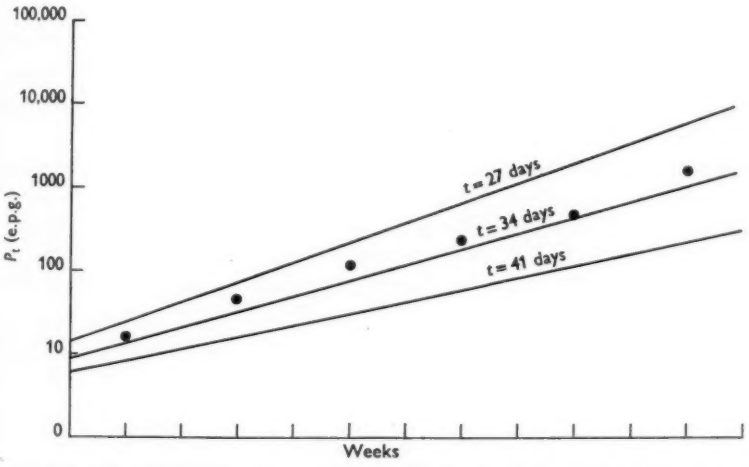

Fig. 1. Values for *Haemonchus contortus* calculated for $E=6$ and $t=27$ days, 34 days and 41 days. Values extracted from Gordon's 1945 Cherry Hill data are plotted on the same scale.

likely to be achieved by selecting a form with a high value for E and a short generation time. *Haemonchus contortus* was chosen despite the fact that this species occurs only in small numbers in the flocks observed. It is a species which has been studied in considerable detail in both Australia and the United States and much data is available. Various values for E were tested by a system of trial and error. $E=6$ was chosen as a reasonable fit with observations made on *H. contortus* locally. To obtain a further test of the validity of this choice, data from Gordon's 1945 Cherry Hill data (Gordon, 1950) was extracted and plotted with the figures calculated from the estimate. Fig. 1 shows that the choice of $E=6$ was not an unreasonable one.

To prevent any suggestion of unconscious bias it was decided to adopt the figures quoted by another worker. The figures chosen (Table 2) were those of Kates (1947),

because they appeared to be the best documented and based on the greatest number of observations.

Table 2. Worm/egg ratios from Kates (1947)

Haemonchus contortus	1:1
Trichostrongylus spp.	5·1:1
Ostertagia spp.	1·8:1
Cooperia sp.	5:1
Nematodirus sp.	18·1:1
Oesophagostomum sp.	1·2:1

The values given by Kates are in fair agreement with estimates of rates of egg production given by Peters, Leiper & Clapham (1941): *Nematodirus* 1; *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Strongyloides* 10; *Oesophagostomum*, *Chabertia*, *Haemonchus*, *Bunostomum* 100. It will be noted that Kates did not give figures for *Bunostomum* or *Chabertia*. For the purposes of calculation it will be assumed that the estimate for *Oesophagostolum* can be applied to these forms. This is an assumption based on the figures of Peters *et al.* and supported by my own observations.

Using these figures for relative rates of egg-production substitution for E (*Haemonchus*) = 6 gives E (*Ostertagia*) = 3·33; E (*Trichostrongylus*) = 1·176; E (*Cooperia*) = 1·2; E (*Oesophagostomum*), E (*Chabertia*) and E (*Bunostomum*) = 5; E (*Nematodirus*) = 0·33.

Applying the formula $P_t = P_0(1+E)^t$ values for P_t can now be computed for different species and different time intervals. For each species three time scales have been applied: first, the minimum theoretical time for each generation; secondly, the minimum time plus 7 days; thirdly, the minimum time plus 14 days. This allows for a wide range of variations in the time of free-living development. The minimum time plus 14 days must approach the maximum mean time of development.

The results of such calculations are plotted in Figs. 2–9. Each of these figures shows the periods when $P_t = 100$, 1000 and 10,000 for each species. By the method of calculation, and as shown by the goodness of fit of the figures for *Haemonchus contortus* with Gordon's Cherry Hill data, values of P_t should represent the worm egg count expressed as worm eggs per gram of faeces. Thus $P_t = 100$ approximates to the value at which an infection would first be detected with any degree of certainty by means of standard egg counting techniques; $P_t = 1000$ implies the presence of the parasite in large numbers; P_t 1000–10,000 covers the range of values in which either spontaneous recovery (self cure) or an outbreak of parasitic disease could occur.

DISCUSSION

The account of the seasonal incidence observed in the flocks studied does not differ in any important respect from the account given previously (Crofton, 1954). Increased sensitivity of the methods has established some important facts: when a flock is considered as a whole all species of nematode parasites found in the flock occur at all times of the year, but the numbers and relative proportions vary con-

siderably throughout the season; all species are represented in the ewe flock throughout the year, but the numbers are small except at the time of the post-parturient rise. The only reservations which must be made about these statements are: a species may be represented in the flock by a very small infection in only one host; they do not apply to species of *Nematodirus*, which must be considered separately.

If the general results and the order of succession are compared with the results of other workers, it will be seen that there are no fundamental differences even though comparisons involve work done on different continents (see work cited above).

The theory described appears to fit the observed data for all species except *Nematodirus* spp. The general applicability of the theory is justified on the grounds that the order of succession of species can be predicted and that any variations can be accounted for without making any other assumptions.

In the case of *Nematodirus* spp. calculations indicate that the increase from $P_t = 1$ to $P_t = 10$ would require about eight generations. This is obviously absurd if the generation time is 42 days or more. If we consider the observed *Nematodirus* season it is clear that initial infections in ewes and lambs occur almost simultaneously in each season and that the period of increase as seen from egg-counts is sufficient for only one complete generation. It is suggested that *Nematodirus* must be considered as a special case and that it can be referred to as a truly semelparous form (see Cole, 1954); that is, there is only one generation with one brood each year. Theoretically a second generation could occur about September in most years, but this has not been observed in the present work. It may be that some special factor is required for the development of each generation and that satisfaction of temperature and humidity requirements is not enough.

The calculated periods for the other species fit the observed data if we allow for the fact that the minimum time for the development of a generation is usually exceeded and that the theory only applies to species in their normal climatic range. Temporary inhibition of development will not affect the general trends except in so far as the mean time between generations is increased. In most circumstances the variation can be defined by plotting curves based on minimum times and minimum times plus 14 days. For all species except *Nematodirus* these curves correspond to the limits of no delay in development and a total delay of 3 months spread over six generations. In most species inhibition of development will be restricted to the free-living period and the length of the prepatent period may be regarded as constant. Variations in the length of the prepatent period of a species in hosts with different immunological histories (see Gibson, 1952, on *Trichostrongylus*) are possible, but as the theory is concerned with lambs in their most susceptible period these will not be discussed here.

Reference to Figs. 2–8 shows that for any given extension of the free-living period the effect will vary from species to species according to differences in rates of multiplication and to the proportionate rather than to the actual extension. Thus in a highly fecund species with a short generation interval (e.g. *Haemonchus*) delays in free-living development will have less effect in altering the time of

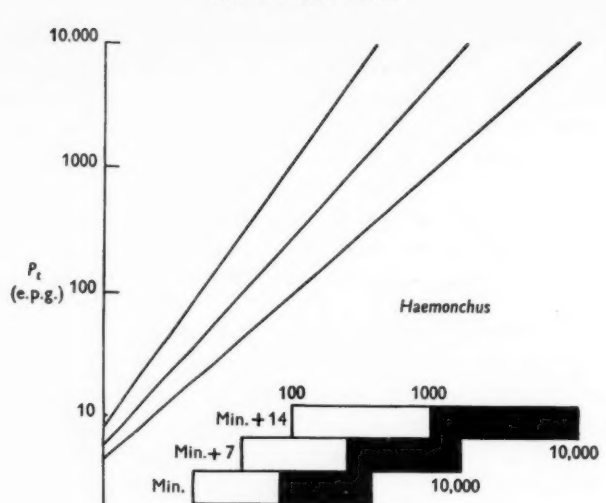

Fig. 2

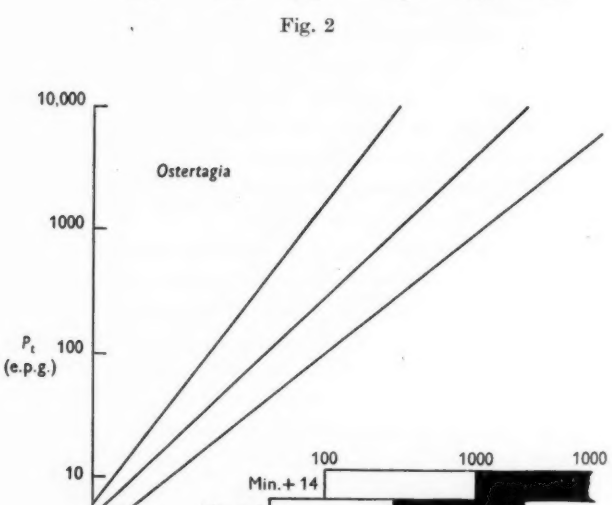

Fig. 3

Figs. 2–9. $P_t = P_0(1+E)^t$ plotted using $E = 6$ for *Haemonchus contortus*, and derived values for the other species. For each species three time scales have been applied: the minimum theoretical time for each generation; the minimum time plus 7 days; the minimum time plus 14 days.

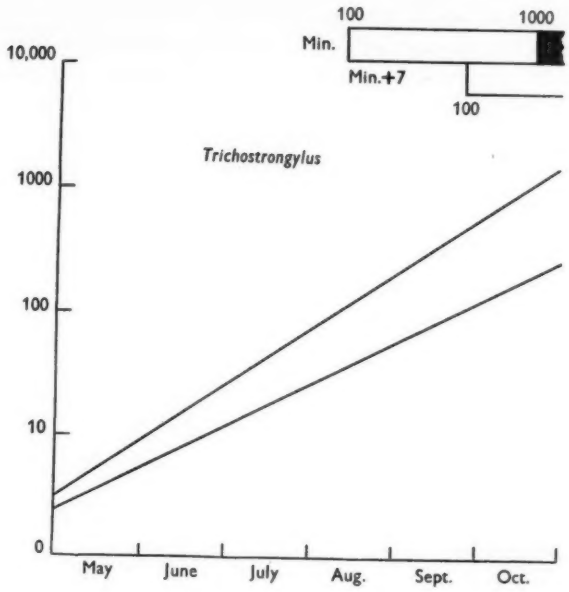
100
1000
Min.
Min.+7
100
10,000
Trichostrongylus
1000
100
10
0
May
June
July
Aug.
Sept.
Oct.

Fig. 4

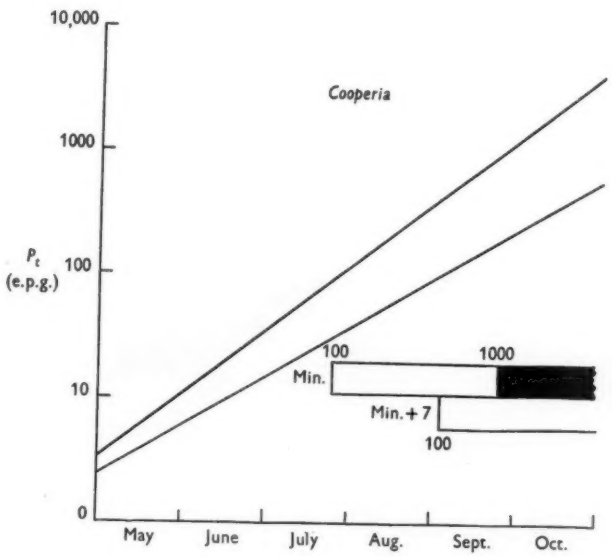
10,000
Cooperia
1000
P_t
(e.p.g.)
100
10
0
100
1000
Min.
Min.+7
100
May
June
July
Aug.
Sept.
Oct.

Fig. 5

achieving a particular level of infection than the same delay in development of a species with a short generation time and a low fecundity (e.g. *Cooperia*). The actual reduction in numbers, however, will be temporarily greater in the more fecund form. In species with a long generation time delays in free-living development will have no marked effect on the general pattern of increase unless the delays are long in relation to the prepatent period. This applies to forms of high fecundity like *Oesophagostomum*, *Chabertia* and *Bunostomum*; in forms with a low fecundity the delays will have even less effect.

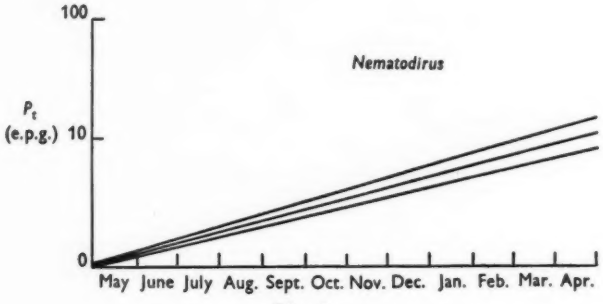

Fig. 6

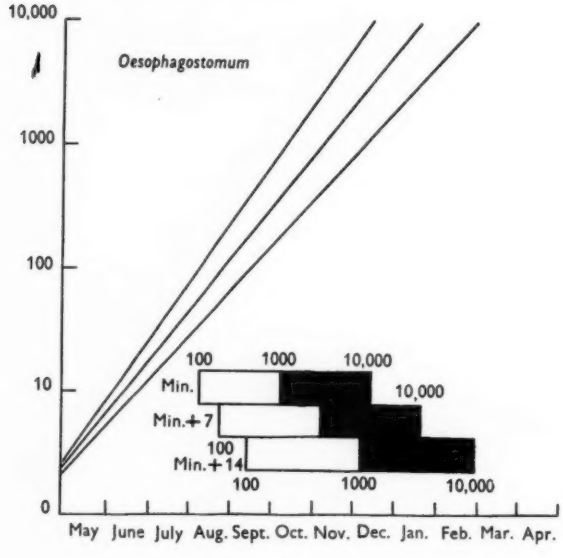

Fig. 7

In general, the ability of free-living stages to survive unfavourable conditions will be essential in forms with a long free-living stage. In most cases, the exception being *Nematodirus*, a long generation time results from a long prepatent period so that variations in the length of the free-living stage are not likely to

upset the basic pattern in these cases. In a species with a short generation time it is unlikely that a period of accumulation of free-living stages would alter the pattern unless a sudden change in infection rate altered the chance of infecting individuals reaching maturity (i.e. produced a change in E).

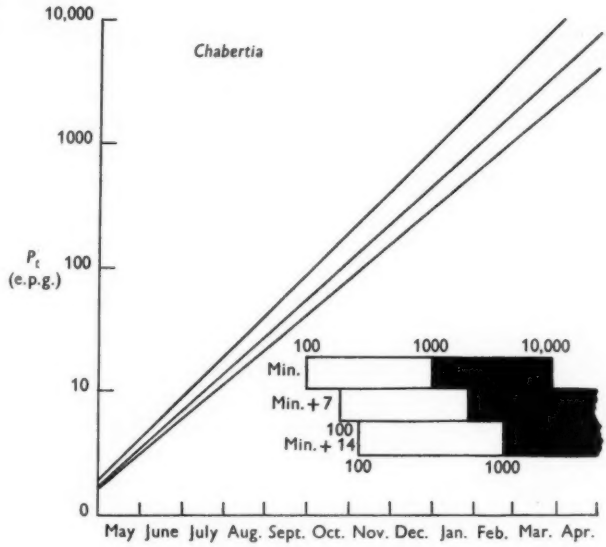

Fig. 8

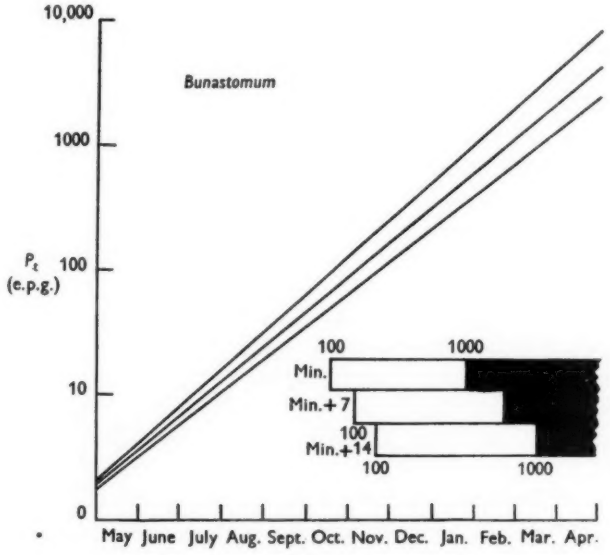

Fig. 9

So far we have considered the effect of changes in length of time between generations (t). The possibility of the value of E changing is a more difficult problem. If, however, we regard E as the *intrinsic rate* of multiplication, some of the greatest difficulties can be avoided. The justification for regarding the intrinsic rate of multiplication as constant during the periods considered is based upon the close approximation of observed increases to a logarithmic pattern. Any significant changes in E would result in an increase which would depart from this pattern. Thus variations in rate of infection may be regarded as due to changes in the length of generation time rather than changes in potential. For the period considered this is probably permissible because the hosts must be in their susceptible phase. The assumption would clearly not be valid in the process of 'self-cure'.

Variations in generation time occur as a result of changes in climatic conditions. It will be noted, however, that such changes will not alter the order of succession of species except under the special circumstances where some species are at the limits of their distribution range. Previously it has been assumed that climatic conditions have been responsible for the regularity of succession. Gordon (1950) stated clearly an opinion shared by many workers: 'The free-living stages on the ground and the herbage have different responses to climatic conditions. In consequence the different species tend to have different seasonal occurrences—a fact which enables the planning of seasonal or strategic control measures.' Clearly this concept of species-succession being a consequence of intrinsic factors rather than ecological ones is novel.

If we regard E as a measure of the *intrinsic* rate of increase then the generation time unit as used here becomes in effect the time required to produce a definite proportionate increase in the egg producing population of the host. In this sense, therefore, factors other than climatic ones can affect the generation time.

Any conditions which reduce the chances of encounter between host and parasite can be regarded as factors which increase the generation time, and most control measures may be regarded in this light. The policy of mixed grazing and rotational grazing are effective in so far as they increase the effective generation times. Changes in stocking rates, by changing the chances of encounter will also affect t. These changes in t are of great importance and on them may depend the ultimate fate of the hosts, because anything which increases t will reduce the rate of increase of the parasite populations. But no method mentioned is likely to have a selective effect upon the species: so that although changes in numbers of parasites may result, the order of succession will not change.

The effect of anthelmintics will be discussed in more detail in a later paper. It is necessary to say here that if an anthelmintic is equally effective against all species, it will not change the order of succession. If, however, the anthelmintic has a selective effect—and this is true of most modern ones—changes in succession could occur, these changes being dependent upon the efficiency and selectivity of the drug used.

As a result of statements and opinions such as those expressed by Gordon (1950) considerable interest has been taken in the effect of climate on the rates of development and time of survival of the free-living stages of nematode parasites. This

interest has been very rewarding in the case of some species, but has contributed little to our knowledge of the epidemiology of others. If we consider the implications of the present theory this is not surprising. It has been shown that the effect of climate on the development and survival of a species can be considered in terms of changes in generation time. These changes will have the greatest effect on the rate of increase of species which are prolific egg-layers and have a short generation time, and least effect on less fecund species with a long generation time. Thus it might be expected that the greatest reward would be obtained by the study of climatic factors in such species as *Haemonchus contortus* and to a great extent this is borne out by examination of the literature. It is no accident that the most important economic forms are the forms which are most likely to be affected by climatic changes because extreme variations in rate of increase of parasite populations will tend to give rise to epidemics.

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THE STRUCTURE OF THE CUTICLE OF *ASCARIS LUMBRICOIDES* VAR. *SUIS*

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(With Plates XIV–XVI)

INTRODUCTION

Investigations into the structure of the cuticle of *Ascaris lumbricoides* have occurred in three phases. The first started in the middle of the nineteenth century (von Siebold, 1845; Czermak, 1852; Bastian, 1866; Leuckart, 1876), the second at the turn of the century (van Bömmel, 1895; Toldt, 1899, 1904, 1905, 1912; Goldschmidt, 1904; Chitwood & Chitwood, 1937), and the third is occurring now with the advent of X-ray diffraction and the electron microscope (Fauré-Fremiet & Garrault, 1944; Picken, Pryor & Swann, 1947; Rudall, 1955).

Of the earlier investigators mentioned, Czermak (1852) undoubtedly contributed the most knowledge. He followed von Siebold in dividing the cuticle into an outer 'epidermis' and an inner 'corion', and appears to have been the first worker to observe that the 'furrows' in the outermost layer tended to end in the lateral lines. Under this layer he observed a fibrillar structure—clearly the fibrillar layer mentioned by later workers. He recognized also the thick homogeneous layer, two fibre layers crossing at an angle of 45°, and appears to have been the first to recognize the basal lamella with its fine striations directed along the longitudinal axis.

Czermak examined also transverse and longitudinal sections under polarized light, and reported the presence of birefringence in most of the layers which he studied.

Neither Bastian (1866) nor Leuckart (1876) was able to contribute much more to Czermak's description.

Almost twenty years later, van Bömmel (1895) investigated the cuticles of several ascarids in detail, and reported the presence of nine layers in the cuticle of *A. lumbricoides* and *A. megalocephala*. These nine layers, which are recognized by Chitwood & Chitwood (1937) and demonstrated in the present work, are: (1) outer cortical, (2) inner cortical, (3) fibrillar, (4) homogeneous, (5) boundary, (6) outer fibre, (7) middle fibre, (8) inner fibre, (9) basal lamella.

Van Bömmel recognized two different layers in the outer cortical layer, viz. a thin, strongly refractive outer layer and a thicker inner layer, the former being divided into ribbons by the grooves on the external surface of the cuticle. He mentions 'circular lamellae', which break up into granular fibres and go as far as the fibrillar layer, but he did not think that they were actually connected. He thought that the fibrillar layer consisted of granules and anastomosing processes, some of which penetrated the homogeneous layer and disappeared into the boundary layer. This boundary layer was constricted at intervals corresponding with the constrictions in the outer cortical layer.

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The three fibre layers were described as flattened, diagonal, parallel fibres, the middle layer being at an angle of 45° to the outer and the middle. The innermost layer of the cuticle was termed the basal lamella by van Bömmel, and when viewed from above was seen to consist of fine striations directed towards the longitudinal axis.

Toldt (1899), who studied the structure of the cuticle of *A. megalocephala* and later (Toldt, 1912) that of *Ascaris lumbricoides*, found the two to be identical. Toldt thought that the cuticle was a cellular secretion which was penetrated by a system of secretion tracts or 'Saftbahnen' which ran from the subcuticle to the surface. He thought that van Bömmel's circular lamellae and their fibres and the fibrillar layer and its processes were all part of this system, and he presumed that the function of the 'Saftbahnen' was for the feeding and preservation of the cuticle from the subcuticle.

Toldt recognized all the layers mentioned by van Bömmel except the fibrillar layer, but instead of a single basal lamella he recognized two: i.e. an outer, thicker basal lamella and an inner, thinner limiting membrane or 'Grenzmembran'. He did not consider, as did van Bömmel, that the outer cortical layer consisted of two layers. He thought that the inner cortical layer was probably part of the homogeneous layer, but was more densely packed and that it was separated from the homogeneous layer by a reticulum of very dense 'Saftbahnen' corresponding to van Bömmel's fibrillar layer and considered by Toldt to be a complicated system of 'Saftbahnen'. Toldt thought that the homogeneous layer was a sort of matrix which was penetrated by 'Saftbahnen', which continued on through the fibre layers into the subcuticle.

These observations of Toldt were severely criticized by Goldschmidt (1904), who maintained that such structures do not exist. Goldschmidt agreed with van Bömmel in most aspects, but recognized the innermost border membrane described by Toldt, and he also observes that the fibres which come from the surface between the rings of the cortical layer (van Bömmel's 'circular lamellae') are connected with the fibrillar layer and that, from here, fibrils may penetrate as far as the boundary layer. Goldschmidt states also that the fibrillar layer originates from the fraying of the inner cortical layer.

The most recent investigation into the structure of the whole cuticle of *A. lumbricoides* has been carried out by Chitwood & Chitwood (1937), who are in almost complete agreement with van Bömmel. They do not consider that the internal cortical layer is an actual entity as it is often quite similar in consistency to the homogeneous layer. They consider that the fibrillar layer consists of a 'condensation of this 'spongy matrix', which forms a network between the internal cortical layer and the homogeneous layer, with strands of condensed material extending into the external cortical and boundary layers. The homogeneous layer, which Chitwood & Chitwood term the matrix layer, has the appearance of a spongy mass. The boundary layer, according to Chitwood and Chitwood, is distinct only in some instances and should be interpreted as a 'condensation' layer. The fibre layers and basal lamellae are as described by van Bömmel.

More recently, the fibre layers have received attention from a number of workers

(Fauré-Fremiet & Garrault, 1944; Picken *et al.* 1947), who have shown that the fibres which cross at 135° give a collagen X-ray diffraction pattern, but do not give a characteristic banded collagen structure when examined under the electron microscope (Rudall, 1955).

It is apparent from this review that the more recent investigators differ quite considerably in their views of certain aspects of cuticle structure. Because a knowledge of cuticle structure is an important initial step in the study of cuticle secretion and ecdysis, we have decided to investigate the structure once again.

MATERIAL

Specimens of *A. lumbricoides*, var. *suis* were obtained alive and taken to the laboratory in 'Ringer's' solution (Baldwin & Moyle, 1947) at 38° C. When the cuticle alone was examined it was removed from the worm as previously described (Bird, 1956).

METHODS

Light microscopy

(*a*) *Surface preparations*

Specimens were either examined by focusing through the various layers of fresh cuticle in saline from above or the individual layers were examined after exposure by a slight modification of the layer stripping technique described by Reed & Rudall (1948). The cuticle when freshly stripped from the worm was placed on a clean glass slide with its outermost layer exposed and allowed to dry overnight in a desiccator. One of the edges was then moistened so that the cuticle could be firmly grasped by fine forceps and a strip was torn off. When examined from above, the edges of this strip clearly demonstrate the various layers.

(*b*) *Sections*

Wax sections were cut at 6μ, using a Spencer rotary microtome and were stained in Mallory's triple stain. Frozen sections were cut at 10μ, using a Sartorius–Werke freezing microtome, and these were examined in distilled water or in glycerine jelly under phase contrast and under polarized light.

Electron microscopy (using the Siemens electron miscroscope Elmiscop 1 *at* 80 *kV.)*

(*a*) *Surface preparations*

Specimens of the fibre layer and the basal lamella were prepared by the layer stripping technique of Reed & Rudall (1948). The outermost layer was prepared by a further modification of their technique, in which the cuticle was placed between two glass slides. After drying in a desiccator, the slides were pulled apart and the uppermost side was lightly flooded with a 0·5% solution of collodion in amyl acetate which was quickly tilted and drained. After the solvent had evaporated, the collodion film was floated off on water and transferred to a specimen grid.

(*b*) *Sections*

Portions of cuticle were fixed in either formol calcium or 1 % osmium tetroxide in phospate buffer at pH 7·2 for 30 min. and then dehydrated in ethanol: 70 and 96 %, 1 hr in each, followed by absolute ethanol for 2 hr. (two washes). They were then washed once *in vacuo* for an hour in a mixture of 95 % butylmethacrylate/5 % methylmethacrylate. This procedure was repeated, and then the monomer was polymerized at 45°, using benzoyl-peroxide as a catalyst.

Unfortunately, the formation of bubbles caused great difficulties which were not eliminated by careful drying of the monomer and the specimen, although they were reduced by extracting *in vacuo* for 2 hr. It is possible that longer treatment *in vacuo* might completely eliminate these bubbles, which are derived from the cuticle.

Sections were cut at about 250 Ångström units, using glass knives on a Porter–Blum microtome. The cuticle was difficult to cut and the sections tore easily and tended to form folds. Although this part of the investigation was only of a preliminary nature, it was thought that the results were interesting enough to be included in this paper.

RESULTS

(1) *Surface preparations*

An examination of the surface layer, by focusing directly on to the cuticle from above, clearly shows the transverse grooves or furrows first mentioned by Czermak (1852). These grooves are almost twice as far apart in the posterior as in the anterior region. It can be seen (Pl. XIV, fig. 1) that, in the region of the lateral line, they tend to end, but some pass right across. Electron micrographs of this region often show a dense population of small, rod-like bacteria in the grooves (Pl. XIV, fig. 2). These organisms have been isolated in pure culture. They are very small, Gram-negative, motile rods, which produce pyocyanin on nutrient agar at 20° C. and probably belong to the species *Pseudomonas aeruginosa*. Epps, Weiner & Bueding (1950) have described two Gram-negative rods isolated from *Ascaris lumbricoides*, but did not identify them.

The area between the grooves shows ridges which run parallel to the grooves; the grooves themselves are not ridged (Pl. XIV, fig. 3). These ridges are irregular and are generally several hundred Ångström units apart (Pl. XIV, fig. 4).

Focusing from above through to the fibre layers shows that these criss-cross in a characteristic fashion (Pl. XIV, fig. 5).

Layer stripping reveals the presence of the external and internal cortical layers, the homogeneous layer, three fibre layers and the basal lamella. The angle of 135° at which the fibres cross (Picken *et al.* 1947) can be seen clearly (Pl. XIV, figs. 6, 7). An electron microscope examination of the fibre layer, obtained by layer stripping, shows that they do not possess the characteristic banding of collagen fibres. An electron microscope examination of the basal lamella, obtained by layer stripping after it had been initially dissected away from a fresh cuticle, shows that this layer is similar but has a finer structure.

(2) *Sections*

Longitudinal, transverse and tangential wax and frozen sections, fixed and unfixed, have clearly demonstrated the presence of the nine layers originally discovered by van Bömmel (1895). These are most clearly seen in longitudinal section (Pl. XIV, fig. 8).

The structure of the cuticle may be more easily understood if it is examined as a three-dimensional diagram (Pl. XV, fig. 9). The boundary layer has been omitted from this diagram because it is difficult to recognize in some sections.

An electron micrograph of a longitudinal section in the region of the external cortical layer indicates that the surface of the cuticle is covered with a layer less than a thousand Ångström units in thickness (Pl. XV, fig. 10). This layer is osmiophilic and may thus be 'lipoid'. The external cortical layer is clearly visible and shows striations perpendicular to the surface. These perpendicular striations are not visible when the external cortical layer is viewed under oil immersion using phase contrast in fresh unfixed frozen sections (Pl. XV, fig. 11), nor are they visible under the electron microscope when the cuticle is fixed in formal-calcium (Pl. XV, fig. 12). However, there is considerable agreement between electron micrographs showing longitudinal sections of the inner cortical layer (Pl. XV, fig.13) and the same region in fresh frozen sections under phase contrast (Pl. XV, fig. 11). Opening under the transverse grooves are lines of structures arising from the 'fibrillar layer' which resemble dots when viewed in tangential section. Closer inspection of some of these dots reveals that they are circular and appear to be hollow and to have roots radiating away from them at a greater depth (Pl. XV, fig. 14).

These structures are those which were known as 'circular lamellae' by van Bömmel (1895), 'Saftbahnen' by Toldt (1899), 'fibres' by Goldschmidt (1904) and 'strands of condensed material' by Chitwood & Chitwood (1937). Near the surface, they have a characteristic radiating structure which is clearly visible in the light and electron microscopes in longitudinal section (Pl. XV, figs. 11, 13) and, when examined at high magnification under the electron microscope, these radiating structures appear to link up with the previously mentioned perpendicular striations in the external cortical layer (Pl. XV, fig. 10).

The 'fibrillar layer' appears in transverse sections, but its extent depends on where the section is cut, e.g. between the grooves or close to a groove.

As the structures arising from the 'fibrillar layer' are never quite vertical and tend to slope, it is quite impossible to cut thin transverse sections showing them and the 'fibrillar layer', although the top parts of one row may be seen above the 'fibrillar layer' associated with another row. Extensions of the 'fibrillar layer' may penetrate the homogeneous layer (Pl. XIV, fig. 8), although their penetration through the fibre layer, as claimed by Toldt, has not been observed.

The homogeneous layer presents a most interesting and characteristic appearance when it is viewed under the electron microscope (Pl. XVI, fig. 17). It is clearly not homogeneous but the name is retained because it certainly seems to be so when it is viewed under the highest powers of the light microscope. It shows radial striations and these bear a marked resemblance to the ridges between the grooves

on the surface of the cuticle. Unexpectedly, this layer does not exhibit any marked bi-refringence, and the scarcely perceptible amount which was observed could not be correlated with the pronounced striations observed under the electron microscope. These striations appear to pass through the inner cortical layer only in the region of the structures arising from the 'fibrillar layer'. After passing through this layer, they branch out and link up with the vertical striations in the external cortical layer.

The boundary layer can be seen in both transverse and longitudinal sections, but can be seen more clearly in the latter, where it forms a type of segmentation which parallels that of the external cortical layer (Pl. XIV, fig. 8), especially in the anterior region. This layer has not been identified by layer stripping methods, and is difficult to see in some sections. It may be seen together with the fibre layers when examined under polarized light (Pl. XVI, fig. 18).

The orientation of the fibre layers can be demonstrated under polarized light (Pl. XVI, fig. 19), and it is clear from an electron micrograph of a longitudinal section cut through the three fibre layers (Pl. XVI, fig. 20) that the direction taken by the middle layer is different from that of the inner and outer layers. This section and a frozen longitudinal section examined under oil immersion phase contrast (Pl. XVI, fig. 21) show that these fibres are rather irregular in shape and do not appear to be made up of fibrils. They seem to be surrounded by thin membranes, but these may well be an artifact.

The orientation of the basal lamella is best seen under polarized light in longitudinal section (Pl. XVI, fig. 19). In transverse section (Pl. XVI, fig. 22), it can be seen that this layer has radial striations. This particular photograph also demonstrates the relationship of the subcuticle and lateral line with the basal lamella.

DISCUSSION

Whilst the part of this investigation undertaken at the level of the light microscope has not revealed any new layers, it has cleared up several controversial points concerning this complicated structure. This is primarily because freshly cut frozen sections, when observed under phase contrast, show much more structure than stained wax sections do. For instance, it is clear that the inner cortical layer is structurally different from the homogeneous layer, and that Chitwood's fibrillar layer consists of distinct branches which join to form structures, which we suggest might appropriately be termed pore canals as there is some evidence, not yet conclusive, that these are duct-like structures. This name would imply that these structures are involved in the secretion of the cuticle or in its maintenance, as was suggested by Toldt (1899). It is quite possible, particularly in the light of what is now known about the secretion of arthropod cuticle, that the structures arising from the 'fibrillar layer' are pore canals of which the 'fibrillar layer' forms the roots; but physiological or histochemical, as well as further structural, evidence will surely have to be provided before this can be proved, and meanwhile it is better not to introduce a new term. Chitwood's hypothesis that the various layers of the cuticle are formed as protoplasmic condensations in the exterior subcuticle

or hypodermis is difficult to reconcile with its structure, and with what is now known about cuticle secretion and moulting in other invertebrates.

The hypothesis is tentatively put forward, purely on morphological grounds, that the cuticle is secreted by the subcuticle or hypodermis and that the lateral lines are involved, because the external grooves, under which the structures arising from the 'fibrillar layer' open, end in this region in the majority of cases and focusing downwards from above gives the impression that they bend down into the cuticle at right angles to the surface. It is difficult to obtain either transverse sections in the lateral line region along the middle of a groove or longitudinal sections along the centre of the lateral line. Transverse sections do, however, show that the boundary layer becomes pyramidal in this region and the fibre layers are displaced. Some unfixed frozen sections have shown surprisingly regular fibre-like structures, which originate at the boundary layer and run vertically as far as the 'fibrillar layer'.

The lateral lines run the whole length of the worm. In contrast with the rest of the subcuticle they contain nuclei and penetrate inwards towards the alimentary tract of the worm. Fats and glycogen are also stored in this region, which is what one would expect in a region of high metabolic activity.

Investigation with the electron microscope has revealed the presence of several interesting structures. First of all a thin outer, osmiophilic membrane about a thousand Ångström units thick has been observed. This type of structure has been predicted by Trim (1949) on the basis of his experiments on the kinetics of the penetration of certain anthelmintic compounds into *Ascaris lumbricoides*.

The vertical striations in the external cortical layer end as thickenings in the osmiophilic membrane. These striations appear to link up with the radiating, aster-like striations in the outermost part of the structures arising from the 'fibrillar layer' and continue either through or between them and link up with the corresponding radial striations of the homogeneous layer. Formal-calcium-fixed longitudinal sections through this region do not show the radial striation in the external cortical layer.

The inner cortical layer can be seen in longitudinal sections to be made up of striations which radiate downwards from the aster-like structures in the anterior part of the structures arising from the 'fibrillar layer' and form curved striations which run at right angles to the homogeneous layer with their convex faces pointing inwards.

The radial striations of the homogeneous layer appear to penetrate the boundary layer and part of the fibre layers. It is not possible to say whether this penetration goes as far as the subcuticle.

Electron microscopy of the fibre layers and the basal lamella has not so far revealed structures which cannot generally be equally well observed under the light microscope, although it can be shown that no typical collagen periodicities are present in either of these structures, thus confirming previous observations by Rudall (1955) for the fibre layer. In longitudinal section the fibres are seen to be irregular in shape and do not appear to have an internal fibrillar structure.

Further electron microscope studies involving tangential and transverse sections should help to clarify this interesting structure.

A detailed knowledge of structure is essential if the physiological processes of secretion and moulting are to be properly understood.

SUMMARY

1. Nine layers have been identified in the cuticle of *Ascaris lumbricoides* var. *suis* under the light microscope and their nomenclature is identical with that of van Bömmel.

2. The inner cortical layer consists of a distinct structure which is seen to be markedly different from the homogeneous layer when fresh frozen sections are examined under phase contrast.

3. Examination of the outer surface of the cortical layer under the electron microscope after layer stripping and sectioning has revealed the presence of a ridged osmiophilic layer which is less than a thousand Ångström units thick.

4. Longitudinal osmium-fixed sections, when examined under the electron microscope, have shown that the external cortical layer has radial striations which appear to link up with the striations which radiate from an aster-like process at the top of the structure arising from the 'fibrillar layer'.

5. The osmium-fixed homogeneous layer, when viewed under the electron microscope, is seen to consist of fine radial striations.

6. The fibre layers do not show any finer structures when they are examined under the electron microscope. In longitudinal section, they are seen to consist of irregularly shaped fibres without an internal fibrillar structure. Layer stripping shows that they lack the type of periodic structure seen in collagen.

7. The basal lamella is similar, in that the electron microscope does not reveal any finer structures.

We wish to thank Drs P. Mitchell and C. Rayski of this Department for their advice and criticism. We are also grateful to Dr J. M. Mitchison for his assistance with, and advice about, the polarizing microscope, to Miss J. Moyle for culturing the rod-like bacteria and to Mr R. A. Fox for his considerable assistance and advice on photography. The parasite studied was obtained through the courtesy of the veterinary authorities at the Slaughterhouse, Gorgie. We also wish to acknowledge the Melville Trust, which provided a grant to equip the electron microscope laboratory in this department.

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EXPLANATION OF PLATES XIV–XVI

PLATE XIV

Fig. 1. Surface view, showing the arrangement of the grooves in the region of the lateral line.

Fig. 2. Electron micrograph of a surface preparation, showing a dense population of small rod-like bacteria in the grooves.

Fig. 3. An electron micrograph, showing the grooves without bacteria.

Fig. 4. Higher powered electron micrograph, showing the striations between the grooves.

Fig. 5. Focusing through the cuticle from above to show the criss-crossing of the fibre layers.

Fig. 6. Surface view, after layer-stripping, showing, *e.c.l.* (external cortical layer), *i.c.l.* (internal cortical layer, *h.* (homogeneous layer), *o.f.l.*, *m.f.l.* and *i.f.l.* (outer, middle and inner fibre layers), *b.l.* (basal lamella).

Fig. 7. Higher powered surface view, after layer stripping.

Fig. 8. Longitudinal fresh frozen section, 10μ, under phase contrast, showing all layers.

PLATE XV

FIG. 9. Diagram showing transverse, longitudinal and trangential sections. *e.c.l.* (external cortical layer), *i.c.l.* (internal cortical layer), *fi.* (fibrillar layer), *h.* (homogeneous layer), *f.* (fibre layers), *b.l.* (basal lamella).

Fig. 10. Electron micrograph of an osmium-fixed longitudinal section in the region of one of the transverse grooves, showing the external cortical layer and a section through several bacteria in the groove.

Fig. 11. Longitudinal fresh unfixed frozen section, 10μ, oil immersion, phase contrast, showing cortical and 'fibrillar' layers.

Fig. 12. Electron micrograph of a formal-calcium-fixed longitudinal section, showing absence of vertical striations in the external cortical layer.

Fig. 13. Electron micrograph of an osmium-fixed longitudinal section, showing radiating aster-like structure and its connection with the cortical layers.

Fig. 14. Tangential fresh, unfixed, frozen section, 10μ, oil immersion, phase contrast, showing the lines of dot-like structures arising from the 'fibrillar' layer.

PLATE XVI

Fig. 15. Transverse osmium-fixed, frozen section, 10μ, phase contrast, showing a section cut between the grooves. *e.c.l.* (external cortical layer), *i.c.l.* (internal cortical layer), *fi.* ('fibrillar' layer), *h.* (homogeneous layer), *f.* (fibre layers).

Fig. 16. Similar to fig. 15, but showing a section cut close to a groove, which shows part of the 'structures arising from the fibrillar layer'.

Fig. 17. Electron micrograph of an osmium-fixed longitudinal section, showing part of the homogeneous layer.

Fig. 18. Longitudinal formal-calcium fixed, frozen section, 10μ, under polarized light, showing 'structures arising from the fibrillar layer', boundary and fibre layers, × 580.

Fig. 19. Similar to fig. 18, but showing internal cortical, 'fibrillar' and fibre layers (inner and outer) and the basal lamella, × 580.

Fig. 20. Electron micrograph of an osmium-fixed longitudinal section, showing the three fibre layers.

Fig. 21. Longitudinal formal-calcium fixed, frozen section, 10μ, oil immersion, phase contrast, showing the whole cuticle and in particular the three fibre layers with the boundary layer on top and the basal lamella below; part of the subcuticle can be seen adhering to the bottom of the basal lamella.

Fig. 22. Transverse wax section, 6μ, Mallory's triple stain, showing all the layers and the subcuticle and lateral line. *e.c.l.* (external cortical layer), *i.c.l.* (internal cortical layer), *fi.* ('fibrillar' layer), *f.* (fibre layers), *b.l.* (basal lamella), *sc.* (subcuticle), *l.l.* (lateral line), *m*, (muscle).

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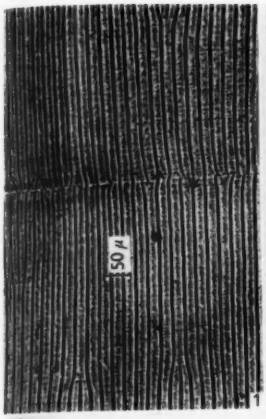

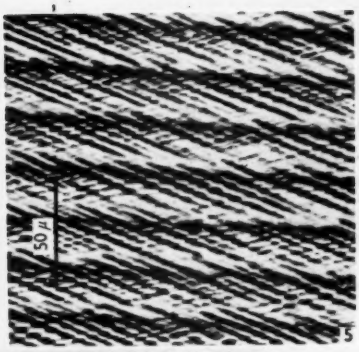

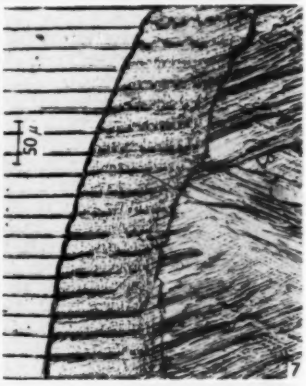

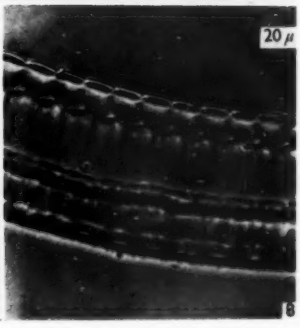

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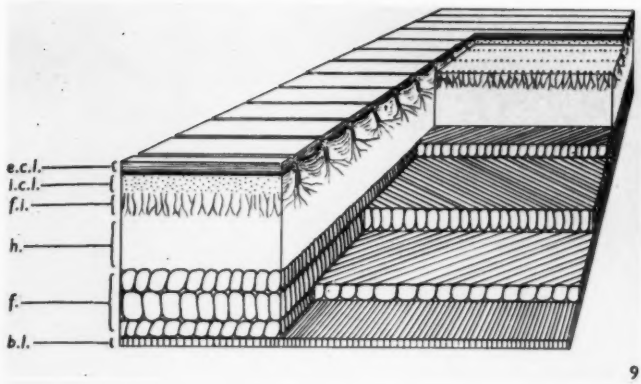

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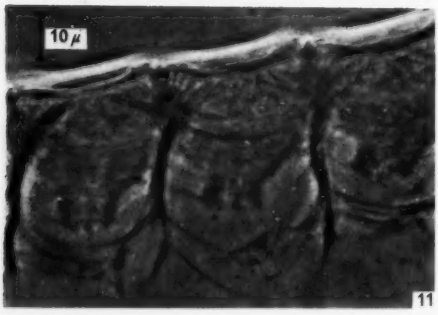

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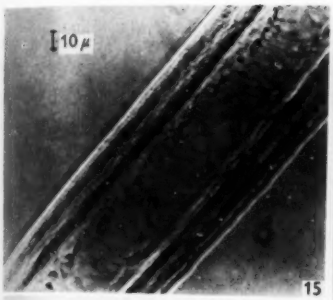
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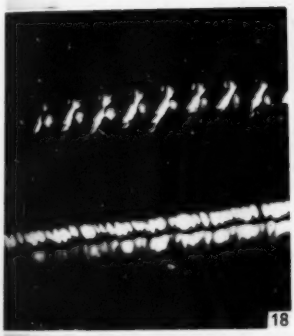
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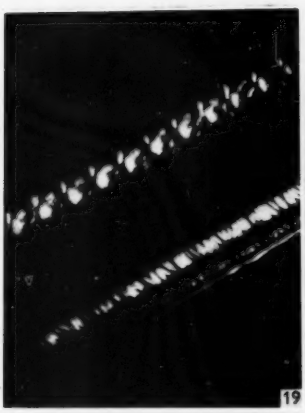
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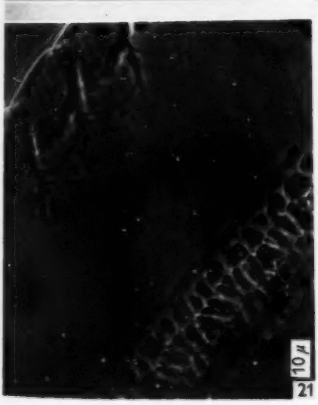
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TWO NEW PARASITIC MITES (ACARINA) FROM THE SOUTH AFRICAN PORCUPINE

By W. M. TILL

Department of Entomology, South African Institute for Medical Research, Johannesburg

(With 6 Figures in the Text)

Three new parasitic mites—a fur mite, a mange mite, and a nasal mite—were recovered recently on dissolving the head of a specimen of *Hystrix africaeaustralis* Peters, the South African porcupine, in a solution of potassium hydroxide. The fur mite is being studied by Dr R. F. Lawrence of the Natal Museum. The other two species, the mange mite, belonging to the genus *Psorergates* Tyrrell, and the nasal mite, belonging to the genus *Rhinophaga* Fain, are described below.

Mites living in the respiratory tract of monkeys, baboons, dassies and dogs were formerly all placed in the genus *Pneumonyssus* Banks (see Zumpt & Till, 1954). Recently, Fain (1955*a*) restricted *Pneumonyssus*, type *P. simicola* Banks, to those species living in the lungs of monkeys, baboons and dassies and proposed a new genus, *Pneumonyssoides*, for the dog parasite, *Pneumonyssus caninum* Chandler & Ruhe. Later the same author (Fain, 1955*b*) erected the new genus *Rhinophaga*, type *R. cercopitheci* Fain, for mites from the nasal cavities of several species of *Cercopithecus* and also included in this genus *Pneumonyssus dinolti* Oudemans from the nasal cavities of *Macaca mulatta* Zimmerman and *Rhinophaga papionis* Fain from the nasal cavities of *Papio doguera tessellatus* Elliot. Both Dr Zumpt of the Department of Entomology, S.A.I.M.R., and the present author are in agreement with Fain's classification.

Psorergates hystrici n.sp.

In the key to the *Psorergates* species given by Zumpt & Till (1955), *P. hystrici* runs down to *P. cercopitheci*, from which, however, it is easily distinguishable. In the male *P. hystrici* the penis is long, extending from a point between the levels of the second and third legs to the anterior margin of the dorsal plate, whereas in *P. cercopitheci* the penis is very short and lies at the level of the second pair of legs. In both sexes of *P. hystrici* the loop formed by the first pair of apodemes is broadly open, whilst in *P. cercopitheci* it is almost closed. In *hystrici*, two pairs of dorso-lateral palpal hairs can be distinguished, the posterior one being a little longer than the anterior one. In *cercopitheci*, the posterior 'hair' appears to be represented only by a triangular, chitinous base. These palpal hairs, however, are very difficult to see.

Male (Fig. 1). Body length (including mouthparts) of holotype 140μ, of four paratypes 118–132μ. Width (between second and third pairs of legs) of holotype 122μ, of paratypes 110–118μ. The dorsal plate is almost circular in shape with a straight anterior margin. In the holotype it is 96μ long and 90μ wide and in the

paratypes the length varies between 90 and 98μ and the width between 88 and 90μ. No setae can be distinguished on the plate. The outer, uncovered part of the dorsum is finely and circularly striated. The penis, which is situated dorsally, extends from a point between the levels of the second and third pairs of legs to the anterior margin of the dorsal plate. On the ventral surface only the first pair of apodemes can be distinguished. A median tubercle near the posterior end of the body bears a pair of long (60μ) slender hairs.

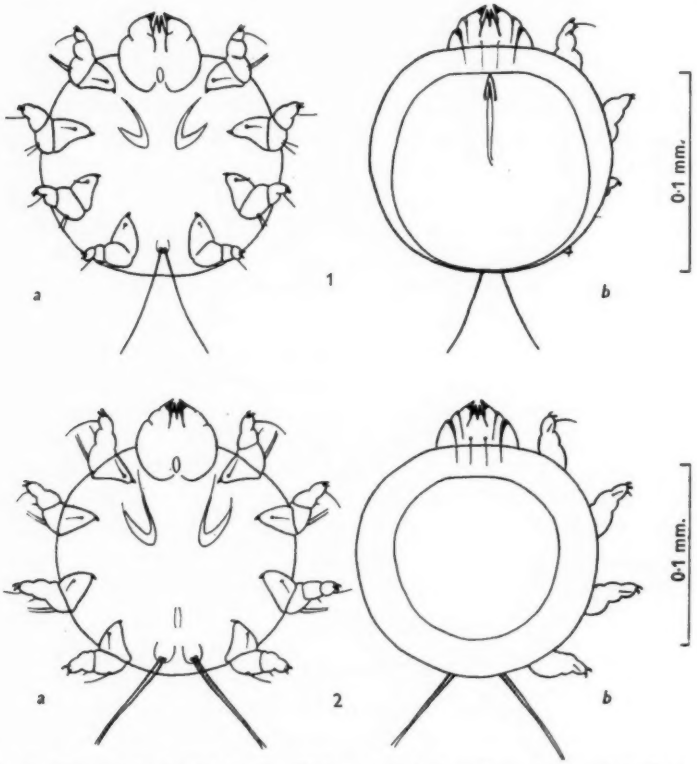

Fig. 1. *Psorergates hystrici* n.sp. Male: *a*, ventral surface; *b*, dorsal surface.
Fig. 2. *Psorergates hystrici* n.sp. Female: *a*, ventral surface; *b*, dorsal surface.

Each palp bears two long dorso-lateral hairs, and on the dorso-median part of the gnathosome there is a pair of very fine, slender setae.

Female (Fig. 2). Body length (including mouthparts) of ten specimens 140–160μ, width between second and third pairs of legs 120–150μ. The dorsal plate is almost circular in shape with the anterior margin straight, and measures 90–100μ in diameter. A pair of tubercles arising from the postero-ventral part of the body each bear a pair of long (90μ) slender hairs. In all other respects the female closely resembles the male.

The holotype (male) and paratypes (four males and ten females) are in the collection of the South African Institute for Medical Research, Johannesburg.

Rhinophaga hystrici n.sp.

This species, in the female sex, is readily distinguishable from the other three members of this genus in having the basal part of the gnathosome distinctly broader than long. In the general shape of the body it resembles *R. dinolti* and *R. papionis*, *R. cercopitheci* being quite distinct in having an opisthosoma which narrows abruptly posteriorly. Further features which may be used to separate *R. hystrici* from *R. dinolti* and *R. papionis* are as follows: the sternal plate is relatively broader in *hystrici* (length/breadth 1·67) and the lateral processes of the underlying portion are short and bluntly rounded. In the other two species the

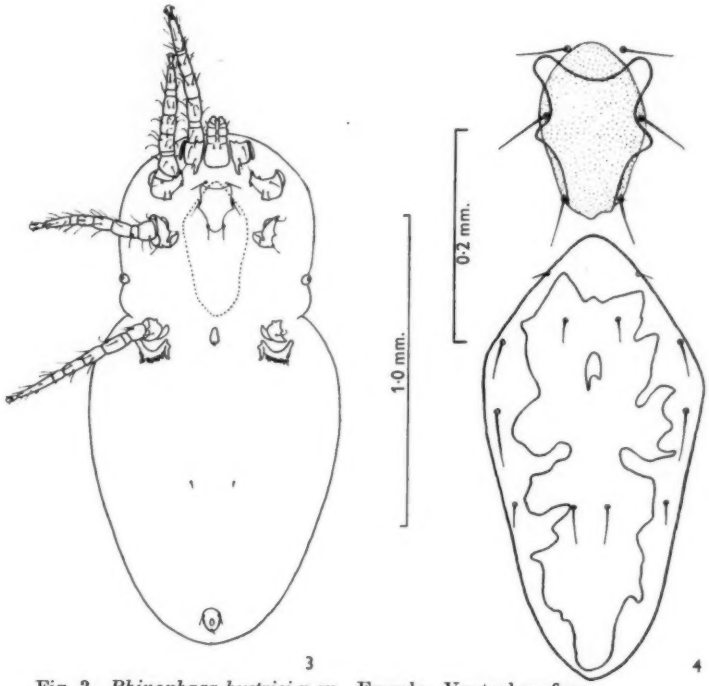

Fig. 3. *Rhinophaga hystrici* n.sp. Female. Ventral surface.
Fig. 4. *Rhinophaga hystrici* n.sp. Female. Dorsal and sternal plate.

sternal plate is twice as long as broad and the underlying processes are long, extending as far as the coxae (cf. Oudemans, 1935, fig. 48; Fain, 1955*b*, fig. A). The hypostome bears three pairs of hairs as in *R. dinolti*, whereas only two pairs are indicated in Fains's drawing of the hypostome of *R. papionis*. The dorsal setae on the distal part of tarsus I are very tiny and difficult to see, but as far as can be determined under oil-immersion, their arrangement is different from that indicated by Oudemans and Fain for *dinolti* and *papionis* respectively.

Female (Figs. 3–6). The body is ovoid in shape with a constriction at the level of the coxae IV. In the holotype the body length (without gnathosome) is 1625μ

and the breadth (between coxae III and IV) is 640μ. In the three paratypes the measurements are 1650 × 648μ, 1725 × 656μ and 1500 × 613μ respectively.

The dorsal plate is roughly ovoid with its greatest width in the anterior third. In the holotype its length is 446μ and its maximum width 210μ. In the paratypes the plate measures 473 × 236μ, 464 × 215μ and 455 × 210μ respectively. There is a median longitudinal thickening, the outline of which is irregular and variable. Of the six pairs of hairs present on the dorsal plate, two pairs are discal and four are marginal. Two pairs of setae can be distinguished on the soft part of the dorsum, one pair lying laterally behind the 3rd coxae and the other pair about one-third the distance from the 4th coxae to the posterior tip of the body and median to the inner margins of the 4th coxae.

The sternal plate is 152μ long in the holotype (150–160μ in paratypes) and 90μ wide at the level of the second pair of sternal setae (90–96μ in paratypes). The plate appears to consist of a superficial part represented in the drawing by the dotted area, and a deeper, more strongly chitinized part which has two pairs of short, blunt, lateral processes behind the 1st and 2nd pairs of hairs respectively. The anterior margin of the plate is not very clearly defined, but the first pair of sternal hairs appear to be situated on the soft skin beyond the margin of the plate. All three pairs of sternal hairs are subequal in length measuring approximately 60μ. The genital orifice is about 300μ wide (265–350μ in paratypes).

The anal plate is 84μ long, 76μ wide and bears a pair of long anterior hairs, a single, shorter posterior hair, and a pair of very minute hairs behind the long anterior ones. These tiny hairs are extremely difficult to discern, and were visible in only one of the paratypes. One pair of setae can be distinguished on the ventral surface of the opisthosoma, about halfway between the 4th coxae and the posterior tip of the body. The peritremes are situated laterally between the 3rd and 4th legs and are 40μ long and 30μ wide.

The leg measurements of the holotype are given in Table 1 (in μ).

Table 1

No. of leg	Total	Coxa	Tro-chanter	Femur	Genu	Tibia	Tarsus	Ambu-lacrum	Width of genu
I	580	90	50	100	80	70	150	40	45
II	505	60	70	90	75	55	110	45	50
III	440	55	60	80	65	55	120	45	45
IV	595	50	60	90	75	80	170	50	40

The chaetotaxy of the legs is as follows: Coxae I–III each bear 2 long ventral hairs, coxa IV has 1 hair. Trochanter I has 3 long hairs and a short internal spine-like bristle, trochanters II and III have 4 and trochanter IV has 3 hairs. Femur I has 8 long hairs and 3 small dorsal spines, femur 2 has 8 long hairs and 2 small dorsal spines, femora III and IV have 5 and 6 long hairs respectively. Each genu bears 8 long hairs. The first tibia bears 8 long hairs, the second 7 long hairs and shorter bristles on the inner ventral surface. Tibia III bears 6 long hairs and one shorter ventral one and tibia IV bears 7 long hairs. Tarsus I has 19 long

hairs plus 13 variously sized setae on the distal part of the dorsal surface (see Fig. 6). Tarsi II and III bear 15, and tarsus IV bears 16 hairs (+ bristles). Unfortunately many of the tarsal hairs were broken so that no statement regarding their lengths can be made.

The gnathosome is 160–170μ long, to the tip of the palps, and has a maximum width of 85–90μ. The basal part of the gnathosome (to level of base of first free palpal segment) measures 65–70μ in length, and is therefore considerably broader than long. The ventral surface of the gnathosome and the chelicerae are shown in Fig. 5.

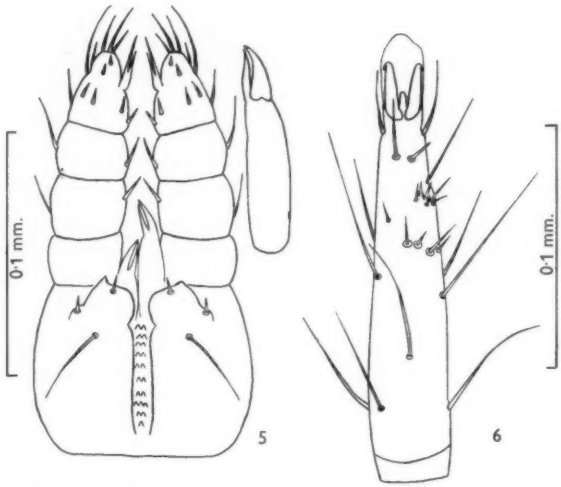

Fig. 5. *Rhinophaga hystrici* n.sp. Female. Ventral view of gnathosome.
Fig. 6. *Rhinophaga hystrici* n.sp. Female. Dorsal surface of tarsus I.

Male. Unknown.

Larva. The body measurements of seven specimens vary from 615μ in length (without mouthparts) by 360μ in width (maximum), to 655 × 395μ. The length of the opisthosoma is less than one-half the body length.

On the dorsal surface five pairs of hairs can be distinguished in the podosomal region. On the posterior third of the opisthosoma there are four pairs of long (50μ) hairs and a pair of lateral (30μ) hairs.

Ventrally, three pairs of hairs ($\pm$ 40μ long) can be distinguished in the sternal region. On the opisthosoma there is a pair of short hairs about one-third the distance from the 3rd coxae to the posterior margin, a pair of longer hairs about two-thirds the distance to the posterior margin, a pair of very long (140μ) hairs near the posterior margin, and a median 70μ hair on the posterior margin. On the posterolateral part of the opisthosoma there are an additional three pairs of short setae. On the hypostome only one pair of hairs can be distinguished with certainty.

The holotype (♀) and three female paratypes are in the collection of the S.A.I.M.R., Johannesburg.

The porcupine was collected near Winburg, Orange Free State, 23 August 1955 and was sent to Dr F. Zumpt by Mr D. H. S. Davis of the Government Plague Research Laboratory.

This paper is a contribution to a survey of arthropods parasitic on vertebrates in the Ethiopian region which is being carried out under the direction of Dr F. Zumpt and with financial assistance from the South African Council for Scientific and Industrial Research.

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THE MORPHOLOGY AND LIFE CYCLE OF *NOSEMA HELMINTHORUM* MONIEZ, 1887*

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(With 39 Figures in the Text)

I. INTRODUCTION

Nosema helminthorum was first discovered by Moniez in 1879 in *Taenia expansa* (=*Moniezia expansa*) and *T. denticulata* (=*M. benedeni*). In 1887 he named the organism and gave a few more details of its structure including the size of the spore. He chose the specific name *helminthorum* believing that the organism was a common parasite of many helminths including *Ascaris mystax* (=*Toxocara cati*). Although Moniez undoubtedly saw most stages of the parasite, he unfortunately gave no illustrations and his descriptions are not detailed. There is little doubt, as I have pointed out earlier (Dissanaike, 1955*b*), that Labbé (1899) was referring to this organism which he reported from the same tapeworms, but he wrongly placed it in the genus *Plistophora*. Weiser (1951) described a microsporidian from a single specimen of *Moniezia benedeni* and gave a few diagrams of developmental stages and spores, calling it a new species, *Nosema bischoffi*, on account of the larger size of its spores; in a previous publication (Dissanaike, 1956) I have shown that he was dealing with the same parasite. Weiser was unfortunate in that he had limited material to work on and so his description too was lacking in certain details.

It would appear from the above that the past accounts of *N. helminthorum* have been both incomplete and inadequate; a detailed description is therefore given in the present work.

II. MATERIAL AND METHODS

The Moniezias studied were obtained from sheep slaughtered in the Metropolitan Cattle Market, Islington. Smears of heavily infected segments were examined in order to study the appearance of fresh spores. Filament extrusion was best accomplished by the mechanical pressure method of Kudo, and the spores with filaments extruded were examined under dark ground illumination. Fresh spores and sporonts were also observed under phase contrast. The various stages of the parasite were studied in dry-fixed smears of infected segments stained with Giemsa's stain, as well as in sections of material fixed in various fixatives and stained most successfully by the Giemsa-Colophonium method. The Feulgen nuclear stain was employed on sections in order to check the number of nuclei in the mature spores, using the technique described by Jírovec (1936). The material was fixed in either Carnoy's fluid or sublimate alcohol acetic acid, the sections being

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hydrolysed in N-HCl for 4–6 min. at 60° C. These preparations were counterstained in 1 % Light Green.

Sections of infected worms showing fusiform cells were also stained in Heidenhain's haematoxylin, Mallory's triple, Mallory's acid fuchsin, Mallory's phosphotungstic haematoxylin, and Van Gieson and Bielschowsky's silver stains, in order to find out the nature of these cells.

III. MORPHOLOGY AND DEVELOPMENT OF THE PARASITE

(*a*) *Early stages and schizonts*

The sporoplasm enters the host tissue where it multiplies by binary or multiple fission. Both these processes have been referred to as schizogony. It was originally thought that there was only one such phase of schizogony in the microsporidia, but in recent years there has been a tendency to define two phases of this process. As both these phases were met with in the present species the description that follows will be given under the headings (i) primary schizogony and (ii) secondary schizogony (autogamy).

(i) *Primary schizogony*

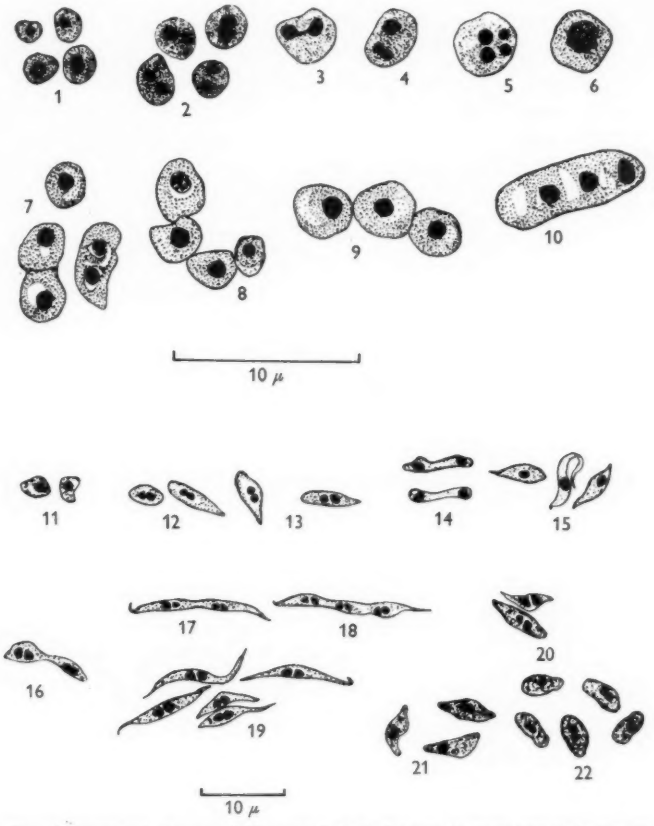
Although a large number of infected worms were studied in detail, developmental stages were seen in only a few; they were mostly absent when the development of the parasite had progressed to the spore stage.

The earliest stage of *N. helminthorum* as seen in Giemsa-stained sections (Fig. 1), is a small spherical body of 1·4μ diameter having a very deeply staining cytoplasm. This stage is generally found quite close to the subcuticular cells. The nucleus is a dense red granule lying in the centre. Later the cytoplasm increases in size and the nucleus divides producing a binucleate body (Fig. 2). Most of these binucleate forms divide into two by simple binary fission, an appearance easily seen in smear preparations. Although actual details of the nuclear division have not been observed, it is clear from Fig. 3 that it is amitotic. The nucleus elongates and divides into two and the nuclear bridge remains between the two for some time. Appearances such as these have been noted in other microsporidia by several workers, such as Stempell (1909) in *N. bombycis*, Fantham & Porter (1912, 1914) in *N. apis* and *N. bombi*, Kudo (1921) in *N. baetis*, and more recently by Canning (1953) in *N. locustae*.

As schizogony proceeds, the cytoplasm begins to stain less deeply and a clear zone appears around the nucleus (Fig. 7). Some of the schizonts do not divide by binary fission, but remain spherical and undivided while the nucleus continues to divide. In this way schizonts with 2, 3 or even 4 nuclei are formed, which probably give rise to daughter schizonts by cytoplasmic cleavage (Figs. 4, 5). Occasionally chains of schizonts are seen (Figs. 8, 9) which are most probably formed by a series of binary fissions, but it is quite possible that such an appearance may result from the cytoplasmic cleavage of an elongate multinucleate schizont (Fig. 10) as shown by Weiser (1951).

In some schizonts that stain less deeply, the nuclei appear less compact and seem to be made up of a number of chromatin granules or threads which occupy a

greater space than the compact nuclei. These nuclei (Fig. 6) bear a strong resemblance to those illustrated by Kudo (1924) for *Thelohania legeri*. The significance of this appearance of the nuclei is uncertain, but it is possible that the schizonts in which they occur are the precursors of the autogamous schizonts which follow.



Figs. 1–22. Developmental stages of *Nosema helminthorum* in sections of tapeworm (Giemsa-Colophonium). 1, Early uninucleate schizonts; 2, early binucleate schizonts; 3, schizont showing nuclear division; 4, 5, tri- and tetra-nucleate schizonts; 6, early schizont showing nucleus composed of several granules; 7, schizonts showing clear zone around nucleus; 8, 9, chains of schizonts; 10, elongate tri-nucleate schizont. 11, early-second-phase schizonts; 12, 13, nuclear division and elongation; 14, nuclear separation; 15, stages resulting from preceding stage; 16–18, division of fusiform schizonts with chain formation and 'diplocarya' formation; 19, fusiform schizonts with double nuclei in close association; 20–22, shortening of processes of fusiform schizonts with deepening of cytoplasm and fusion of nuclei.

(ii) *Secondary schizogony (autogamy)*

In two infected worms a peculiar type of cell was observed in large numbers. The cells were fusiform with long, drawn-out processes, usually with vesicular nuclei containing one or two chromatin granules. These cells bore a strong resemblance to fibroblasts and many tests and stains were carried out to verify their

nature. As will be discussed later, they proved to be secondary schizonts and not fibroblasts. These fusiform cells were seen best in a worm where all stages from primary phase schizonts to spindle-form uninucleate (Fig. 15) and later binucleate cells (Fig. 19) occurred. It is therefore clear that once spore formation is about to commence the schizonts begin to elongate (Figs. 12, 13), their nuclei divide and at first (Fig. 14) become widely separated, after which the cytoplasm constricts between them. This process can continue till a chain of schizonts is formed (Figs. 16–18). Soon the nucleus of each of these cells divides into two but the two halves do not separate (Figs. 17–19), they remain closely associated forming the 'diplocarya' of Debaisieux. The fusiform cells at this stage vary from 7·5 to 17μ in length in sections, and at first are 1·4–1·5μ wide but soon increase in width to about 2μ. The cytoplasm of these fusiform schizonts, which stains faintly at first, becomes more and more deeply stained and vacuolated (Fig. 20). As the cells widen they lose their processes and become at first diamond-shaped (Fig. 21) and later ovoidal (Fig. 22), measuring 6–7·5μ in length with denser, more vacuolated, purplish staining cytoplasm. The nuclei during the whole of this process remain closely associated and gradually come in contact with one another, while in some by the time the ovoidal stage is reached they fuse into a single nucleus, which has a characteristic appearance. The ovoidal stages are clearly the sporonts which are soon transformed into spores.

The second phase of schizogony has thus given rise to elongate sporont mother cells whose nuclei, though divided, do not separate but fuse sooner or later during sporogony. There can be little doubt from these observations that autogamy takes place in this species. It may be that the fusiform cells with elongate processes are not the usual precursors of the sporonts, as they are so rarely met with. One more commonly finds sporont precursors to be faintly staining ovoidal cells resulting from late primary-phase schizonts, but these are found in areas where there are few stages of the parasite together with spores. It is possible therefore that similar nuclear division and fusion also take place here, but it occurs so rapidly that it is not easily made out in these forms.

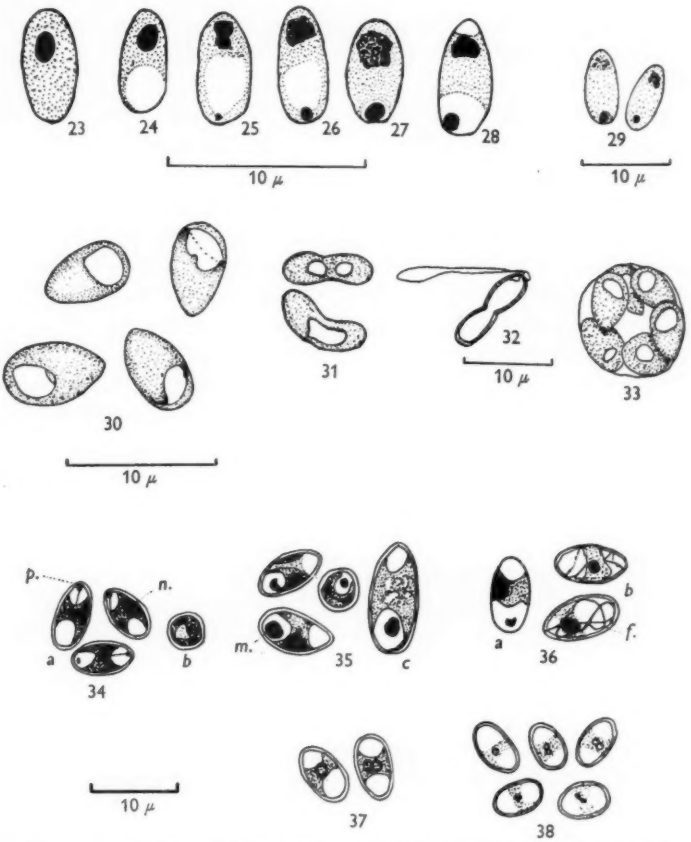
It is not easy to explain why this second phase of schizogony takes on different patterns in different worms and why the fusiform cells with long processes appear only in some. It is of course possible that the continued contraction and relaxation of the worms can account for this morphological character, for in many areas they seem to be most numerous in the region of the well-developed longitudinal muscle bundles of the parenchyma.

(*b*) *Sporogony*

(i) *Sporonts*

In the genus *Nosema* each sporont gives rise to a single spore. In worms in which the fusiform schizonts appear, the sporonts are formed from these elongate cells by shortening of their processes, and, as was mentioned earlier, these sporonts have one or two nuclei according to whether nuclear fusion has taken place or not. In worms that show the larger type of spore and in which the fusiform sporont-precursors are absent, the schizonts give rise to the sporonts by becoming ovoidal

with a centrally placed nucleus and weakly staining cytoplasm. The subsequent development of the two types of sporonts is the same and the description that follows applies to both.



Figs. 23–38. Sporogony of *Nosema helminthorum*. **23–28**, stages in development of the sporont from smear preparation stained in Giemsa stain; **29**, fresh sporonts stained in Toluidine blue; **30**, fresh spores in saline; **31**, double spores in saline; **32**, extruded filament in a double spore, in saline; **33**, six spores within a common membrane in a fresh preparation; **34**, spores from sections stained in iron-haematoxylin, showing polar filament attachment (*p.*) and nucleus (*n.*); **35**, spores stained by Maximow's method showing metachromatic body (*m.*) and macrospore (*c.*); **36**, spores stained in Giemsa (sections), showing filaments (*f.*) after over-differentiating; **37**, spores from sections which showed fusiform schizonts, showing uni- and binucleate stages; **38**, spores from sections stained by Feulgen technique showing various appearances of nuclei.

The sporont has generally a single compact nucleus near the middle of an ovoidal body (Fig. 23). In smear preparations the nucleus may appear to be made up of several chromatin granules (Figs. 26, 27). The structure of the sporont at this stage is best seen in dry-fixed smears, but fresh preparations stained in dilute Toluidine blue or examined under phase contrast show a distinct granule at the posterior pole within a vacuole (Fig. 29); this granule stains a reddish colour with Toluidine

blue and increases in size as the sporont is transformed into the spore. A darkish area in the cytoplasm at the anterior pole represents the nuclear region of the sporont. In Giemsa-stained dry smears these sporonts show at an early stage a clear vacuole at the posterior end (Fig. 24); later on a deep-purple granule appears in this space (Figs. 25–27), which corresponds no doubt to the granules seen in fresh preparations. The granule then increases in size and becomes the metachromatic granule of the mature spore. It is proposed to refer to it at this stage as the 'premetachromatic granule'. This structure has not hitherto been described in the microsporidia but a few observers seem to have illustrated it in the sporonts, although they have not referred to it in any detail. Schröder (1909) shows that the nucleus of *Thelohania chaetogastris* is surrounded by a vacuole, while in the middle of the cytoplasm there is a deeply staining oval body. It is possible that the latter is really the true nucleus. Again Kudo (1925) describes in *Stempellia magna* deeply staining granules in a clear area at one end of the sporoblast. According to him these granules become smaller in size and larger in number as the filament is formed, which he suggests is an indication that they are probably used up in the formation of the filament. Whatever be the true significance of these granules, it is certain that in *Nosema helminthorum* at any rate they are definite and characteristic structures which are represented in the mature spore by a metachromatic body.

(ii) *Spores*

The fresh spores are characteristically egg-shaped with a clear space (vacuole) at the broader posterior pole (Fig. 30). The vacuole occupies about a third of the length of the spore and is in many instances asymmetrically placed. The spores generally measure 5·81–6·8μ by 3·25μ with an average value of 6·25 by 3·5μ. The surface of the spore is smooth and there is no evidence of a bivalve structure.

A common abnormality seen when there is overcrowding is the appearance of a spore of smaller size which may sometimes be rounder than the normal spore. The average measurements for spores from two different worms showing these smaller spores were respectively 5·25 by 3·2μ and 5·6 by 2·9μ. There is no reason to believe that the smaller spores belong to a different species since they are always associated with overcrowding.

Another abnormality was the double spore (Fig. 31) which is apparently formed by the rapid and precocious formation of the spore wall before a binuclear schizont becomes transformed into a sporont. In these double spores the union is obviously by the broader ends as is seen in Toluidine blue stained preparations where the metachromatic staining body, which is normally at the broader end of the normal spore, is adjacent to its partner in double spores. Furthermore, as shown in Fig. 32, these spores possess a filament and probably a sporoplasm, the filament being extruded from the end corresponding to the usual narrow end.

On more than one occasion fresh smears revealed two or more spores within cyst-like structures, and such a structure containing six spores is shown in Fig. 33. This appearance gives the false impression that the spores were formed from the

same pansporoblast, and it was probably this that led Labbé to place the parasite in the genus *Plistophora*.

Although a large number of methods for extrusion of the filaments were tried, only the mechanical pressure method gave good results. The mode of emergence of the sporoplasm and the extrusion of the filament are discussed in another paper (Dissanaike & Canning, 1957). Wet and dry fixed preparations of spores were quite unsuitable for study of the internal structure, especially those stained in Heidenhain's iron haematoxylin, which produces a number of artifacts. On the whole, sections of infected worms are most suitable for study of spore structure. In sections stained in Heidenhain's haematoxylin there invariably appears at the narrow end of the spore a distinct black granule (Fig. 34*a*) from which a thin but distinct filament extends. This filament soon disappears behind the girdle-shaped sporoplasm which is situated at the centre of the spore. This granule is no doubt the attachment granule for the filament which has been observed by many previous workers, for instance in *Nosema bombycis* (Stempell, 1909) and Kudo (1916), *Plistophora macrospora* (Léger & Hesse, 1916), *P. simulii* (Debaisieux & Gastaldi, 1919; Debaisieux, 1928), *Glugea anomala* (Debaisieux, 1919*c*) and *Plistophora blochmanni* (Zwölfer, 1926).

The girdle-shaped sporoplasm which stains very deeply can be recognized as an annular mass in spores that have been sectioned transversely (Fig. 34*b*). In over-differentiated sections the nucleus is seen as an irregular deeply staining area within this girdle. No other structures are visible in these preparations, but occasionally there is a black-staining speck in the posterior pole of the spore which may represent the metachromatic granule which is seen best in sections stained in Toluidine blue and by Maximow's method. In the latter a large number of the spores (Fig. 35) show a bright red body which is either single or made up of several smaller granules. This has been observed in many other microsporidia and it has been called the metachromatic granule.

In sections stained by the Giemsa-Colophonium method most spores stain a deep red and the girdle-shaped sporoplasm takes on a deep purple colour and shows little differentiation, the nucleus being only occasionally seen (Fig. 36*a*). If the same preparations are over-differentiated many interesting features are revealed. In some a red-staining granule can be seen at the broader posterior pole with the nucleus occupying a position within a girdle-shaped sporoplasm; in others (Fig. 36*b*), a red-staining irregularly coiled thread can be seen to occupy the whole of the intrasporal space and is probably the coiled filament that has become disorganized during fixation. That the filament in the above preparations occupies such a wide space is further evidence in favour of the idea that the polar capsule does not exist in these spores (see Dissanaike & Canning, 1957).

In some of the infected worms, especially those which show the fusiform schizonts, the spores are smaller and sometimes rounder; their nuclei may be either single or double, the double ones being in close apposition (Fig. 37). In a few worms there appear spores of both types, the larger spores generally showing a single nucleus, while the smaller spores have either one or two nuclei. It is clear from the above that one cannot distinguish two different groups of spores on the basis of size, for

there exist all transitions from the longer spores to the shorter ones. All these facts further support the view that the spores of different sizes occurring in the different infections are not spores belonging to distinct species, but represent merely morphological variants resulting from variations in the process of schizogony. Kudo (1924*b*) claims that these differences 'are quite probably due among many unknown circumstances to the environmental conditions in the host body—notably the size, nature and condition of the host cell in which the development of the microsporidian took place'.

Finally the nature of the nuclear structure of the spore is confirmed in sections stained by Feulgen's method (Fig. 38).

IV. DISCUSSION

It is necessary first to mention briefly the evidence in support of the fact that the fusiform cells described above are indeed schizonts and not host fibroblasts. Several attempts were made to see if tapeworms kept *in vitro* after injecting indian ink under the cuticle would show a fibroblastic reaction, but this did not occur. Sections of the infected worms that showed these fusiform cells were then stained by various methods to bring out fibrous structures, but all that the staining revealed was that the processes of these fusiform cells were much longer than they appeared in Giemsa-stained sections. These sections were shown to Professor G. R. Cameron, F.R.S., of University College, London, who thought that, if they were definitely not stages of the parasite, their appearance was strongly suggestive of fibroblastic cells met with in vertebrates and higher invertebrates. In a recent demonstration (Dissanaike, 1955*b*) I suggested that these cells were either fibroblasts or aberrant schizonts of the microsporidian, and that in the former case this would be the first report of any histopathological reaction in tapeworms. Since then, however, a thorough study of the literature on the microsporidia indicated that these cells are actually stages of the parasite. In this connexion, the works of Weissenberg (1926) on *N. binucleatum* and Kudo (1944) on *N. notabilis* leave no doubt that the authors met with the same type of cells, which they refer to as spindle-form schizonts and which had previously been seen by many other workers in microsporidia. Both Weissenberg and Kudo describe and depict some of these cells with very long processes not unlike those seen in *N. helminthorum*. Weissenberg regarded them as aberrant schizonts, but their appearance, in the present species at any rate, shows that they are far from abnormal. Kudo's demonstration of similar cells in *N. notabilis* (parasitic in the trophozoites of a myxosporidian *Sphaerospora polymorpha*) confirms that these structures cannot be fibroblasts and clearly proves that they are second-phase schizonts.

The metachromatic bodies seen in the present species were noted by Weiser (1951). They were first observed among the microsporidia in *Plistophora longifilis* by Schuberg (1910), who found that they stained red with Toluidine blue and Polychrome Methylene Blue, and a dark greyish brown with Thionin. He surmised that they were some mucoid material that normally fills the whole intrasporal space and which forms clumps under the influence of fixatives. He also stated that

they probably swell under certain conditions and thereby force the filament out. These bodies were also observed by Weissenberg (1913) in *Glugea anomala* and *G. hertwigi*, where they were seen as large round volutin grains in the posterior vacuole. Zwölfer (1926) saw them in the spores of *Plistophora blochmanni* and noted that in the immature spores they broke up into smaller granules as the spores matured and finally disappeared. He showed that in spores left in tap water for a fortnight, these granules disappeared and concluded that they had something to do with the maturing of the spores. Jírovec (1936), after studying the spores of a number of species of microsporidia by Feulgen staining, stated that these bodies were mistaken for nuclei by many earlier workers and there was no trace of chromatin material in them. He was, however, unable to say what their chemical nature was. In an attempt to elucidate the chemical nature of these bodies I tried out several histochemical tests at the suggestion of Dr A. G. Everson Pearse of the Post-Graduate Medical School, London, but was unable to come to any definite conclusion and so the nature of these bodies remains unsolved.

Although Moniez (1887) believed that transmission takes place through the eggs of the infected worm, as in *Nosema bombycis* of the silk worm, my studies have clearly shown that this is an unlikely mode of transmission, and that a simpler method obtains in nature.

V. SUMMARY

The life cycle of *Nosema helminthorum* proceeds as follows (see Fig. 39).

Emergence of the sporoplasm. The spores swallowed by the vertebrate host (sheep) reach the small intestine where the tapeworm (*Moniezia*) is already present. Here are extruded the filaments with the sporoplasms attached to their tips. These sporoplasms come in contact with the cuticle of the tapeworm at various places, round off and work their way through the cuticle into the tissues of the worm.

Schizogony. They next proceed between the subcuticular cells (3) and start multiplying by binary fission or by multiple division (4, 5, 6, 7). Some of the so-called schizonts are spherical with three to four nuclei (6), while others are elongate plasmodia which give rise to chains of daughter-schizonts by division (7). All these schizonts give rise eventually to faintly staining schizonts in which the nuclei appear to be less compact. Some of these now enter the second phase of schizogony in which elongate fusiform or spindle-shaped cells are ultimately produced (10, 14). The nuclei of these elongate cells divide and move to the opposite poles, and the cytoplasm constricts between them. In this way a chain of fusiform schizonts may be formed (11). The final products of these divisions are fusiform or spindle-shaped cells (11, 15) which are really the precursors of sporonts, in which the nuclei are divided but not separated. These nuclei remain closely associated and eventually fuse either before the sporont stage is reached or later.

Sporogony. The sporonts are ovoidal cells which are generally uninucleate after fusion of the double nuclei of the previous stage. The nucleus is at the centre. Soon a vacuole is formed at the posterior pole, and within it there appears a pre-metachromatic granule (16) which gives rise to the metachromatic body in the mature spore.

Spore formation. A chitinous spore wall is then secreted (17) and the filament is probably formed at this stage in the central axis of the cytoplasm. The rest of the cytoplasm transforms into the sporoplasm, which surrounds the filament like a girdle (18). The spore now becomes mature and is infective to a new host. It is passed out within the gravid segments of the worm and, when it reaches the exterior, is liberated when the segments degenerate. These spores are quite resistant and probably remain dormant during the winter; they are swallowed by *Moniezia*-infected lambs in spring, when the cycle is resumed.

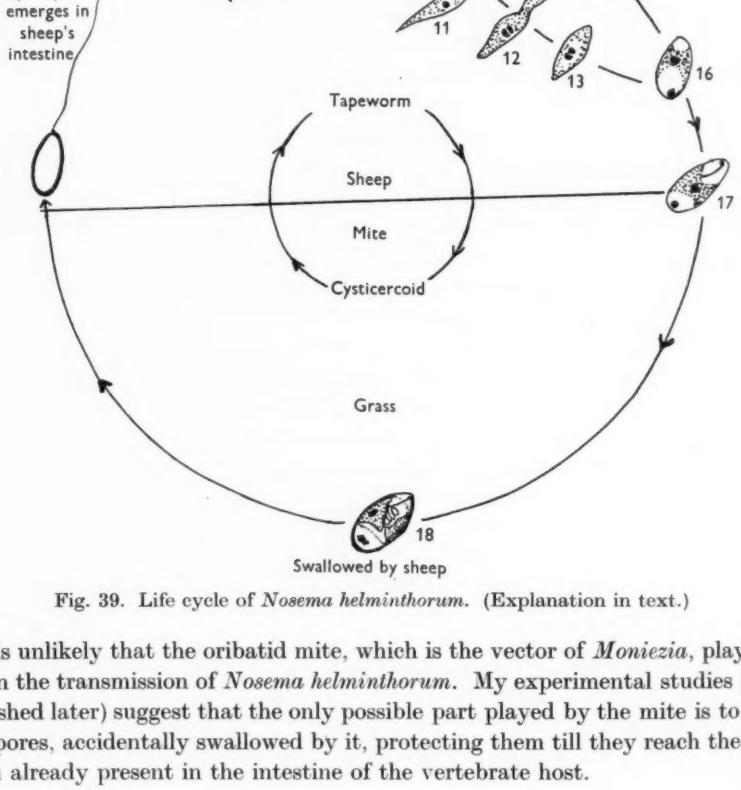


Fig. 39. Life cycle of *Nosema helminthorum*. (Explanation in text.)

It is unlikely that the oribatid mite, which is the vector of *Moniezia*, plays any role in the transmission of *Nosema helminthorum*. My experimental studies (to be published later) suggest that the only possible part played by the mite is to carry the spores, accidentally swallowed by it, protecting them till they reach the adult worm already present in the intestine of the vertebrate host.

I must express my sincere thanks to Professor P. C. C. Garnham for his continued encouragement and guidance in this work, to Professor G. R. Cameron, F.R.S., for

kindly examining my preparations and for his opinions, to Dr A. G. Everson Pearse for help in the histochemical work, to the authorities of the Metropolitan Cattle Market, Islington, for allowing me to collect the material from sheep and especially to Mr H. T. Yelland, Meat Inspector.

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THE MALE OF *IXODES THOMPSONI* ARTHUR, 1955

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(With 7 Figures in the Text)

Ixodes thompsoni was established on three females collected from a cat on Lundy Island (Arthur, 1955). Further collections on Lundy from a cat since that date suggest that the species is relatively common on the Island and we now have the following specimens: cat, Lundy Island, 16. iii. 1955 (1955/81), two females; cat, Lundy Island, 20. iv. 1955 (1955/30), two females with two males in copula; cat, Lundy Island, 31. iii. 1955 (1955/46), five females with four males copulating and one free male.

Description of male. Males are very small, measuring between 1·59 and 1·78 mm. long by 0·76–0·82 mm. broad at about mid-length, and in all cases are brown in colour. The marginal fold is of moderate width and supplied with very long fine hairs, as also is the scutum. Shape elongate oval (Figs. 1, 2).

Capitulum (Figs. 3, 4). Length 0·37 mm., breadth along dorsal ridge 0·17 mm. Dorsal ridge straight; postero-lateral angles sharply pointed; cornua, if present, only very slight; lateral margins either straight or slightly concave, divergent to palpal insertion, more heavily pigmented peripherally. Surface smooth, shining, faintly convex with few small fine punctations. Palps short, broad, articles II and III approximately equal in length, 0·12–0·13 mm.; lateral profile nearly straight, mesial profile convex, apex of article III broadly rounded. Dorsally, hairs along periphery short, two long hairs mesodorsally and proximally on article II. Ventrally, palps with longer hairs; both articles II and III concave on the mesial faces. Ventrally (Fig. 4), posterior margin (when visible) indented in the mid-line, but generally overlapped by strong curved salient ventral ridge, which is continuous with slight (but distinct) projections in the auricular position. Anterior to ridge, surface flat and sloping to hypostomal base, behind the ridge surface declivitous. Widest across auriculae.

Hypostome (Fig. 5). Length about 0·18 mm., strongly idented apically; seven lateral teeth of which the most proximal is lobose and directed ventrally, lateral teeth connected by irregular crenulations which vary from specimen to specimen.

Scutum (Fig. 1). Length 1·1–1·3 mm., breadth 0·64–0·7 mm., widest about mid-length; sides gently curved for most of their length but more sharply angled at the level of the second pair of coxae, postero-lateral and posterior margins more strongly convex. Scapulae short, blunt, prominent; emargination moderate. Cervical grooves distinct, narrow, moderately deep, not reaching back to margins. Surface more strongly curved posteriorly, otherwise generally flattened; divided into two distinct colour zones (indicated by broken line in Fig. 1), the anterior portion more heavily coloured than the posterior. Punctations deep, numerous,

uniformly distributed. Hairs concentrated along the edges and posterior margin, very long and fine, slightly finer and shorter than those on the marginal fold.

Ventral plates (Fig. 2). Pregenital plate with sides tapering anteriorly, posterior border straight, anterior margins irregularly sclerotized but usually with an indentation in the mid-line. Median plate longer than broad; adanal plate about

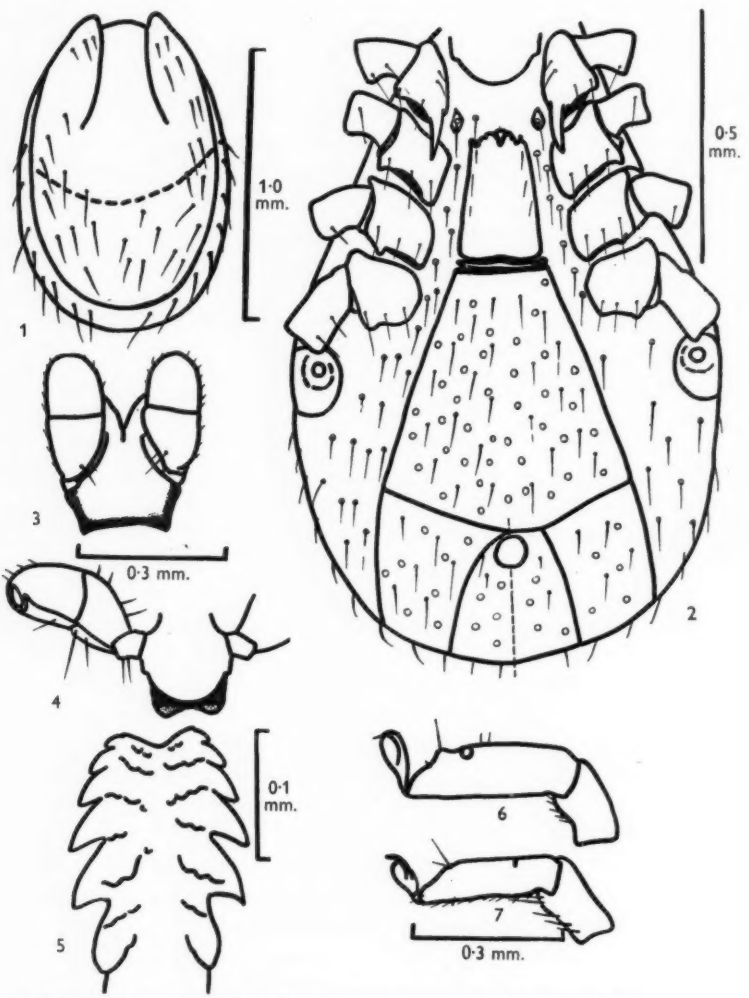

Fig. 1. *I. thompsoni* male. Dorsal view to show scutum and marginal fold.
Fig. 2. Ventral view showing coxae, ventral plates and the variation in form of the adanal and anal plates on either side of the broken line.
Fig. 3. Capitulum—dorsal.
Fig. 4. Capitulum—ventral.
Fig. 5. Hypostome.
Fig. 6. Tarsus I.
Fig. 7. Tarsus IV.

as long as anal plate, latter subject to variation in shape, thus, as on left-hand side of Fig. 2, it may be ogival or (as on the right) be square-ended in front. Where anal plate ogival the adanal plates narrower behind than in front, but where anal plate square-ended in front, the adanal plates roughly rectangular. All plates with punctations similar to those on the scutum; hairy, but generally shorter hairs than on the scutum.

Legs. Short, thick. *Coxae* (Fig. 2). Coxa I with very long, narrow, pointed internal spur which overlaps coxa II, external spur on coxa I short, rounded; external spurs short on coxae II to IV. Coxae I and II with narrow syncoxal areas. Postero-internal angles of coxae II and III may form marginal saliences or such saliences may be lacking. Tarsus I (Fig. 6) short, thick, with sharp drop subapically; tarsus IV (Fig. 7) narrower, with more gradual subapical slope. Length of tarsus I, 0·35 mm., metatarsus I, 0·18 mm., tarsus IV, 0·29 mm., metatarsus IV, 0·18 mm. Pulvillus as long as claw.

Spiracular plate. Subcircular, macula antero-ventral.

Specimens of males are deposited in the collection of G. B. Thompson, the British Museum (Natural History) and in the author's private collection.

I. thompsoni falls into the category of *Ixodes* ticks which have been found *in copula* upon the host (Nuttall, 1911), in which the hypostomes in the male are characterized by prominent basal teeth. It would seem reasonable to believe that these ticks are picked up away from the hosts' nests, probably on the foraging expeditions of the cats. To confirm this it would be highly desirable to examine small mammals on Lundy for this tick, more especially as examination of large numbers of birds from Lundy Island has never yielded this species (Arthur & Thompson, 1953; Thompson & Arthur, 1955, and unpublished data).

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A NEW SPECIES OF *NEOASCARIS* FROM *RATTUS ASSIMILIS*, WITH A REDEFINITION OF THE GENUS

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(With Plate XVII and 10 Figures in the Text)

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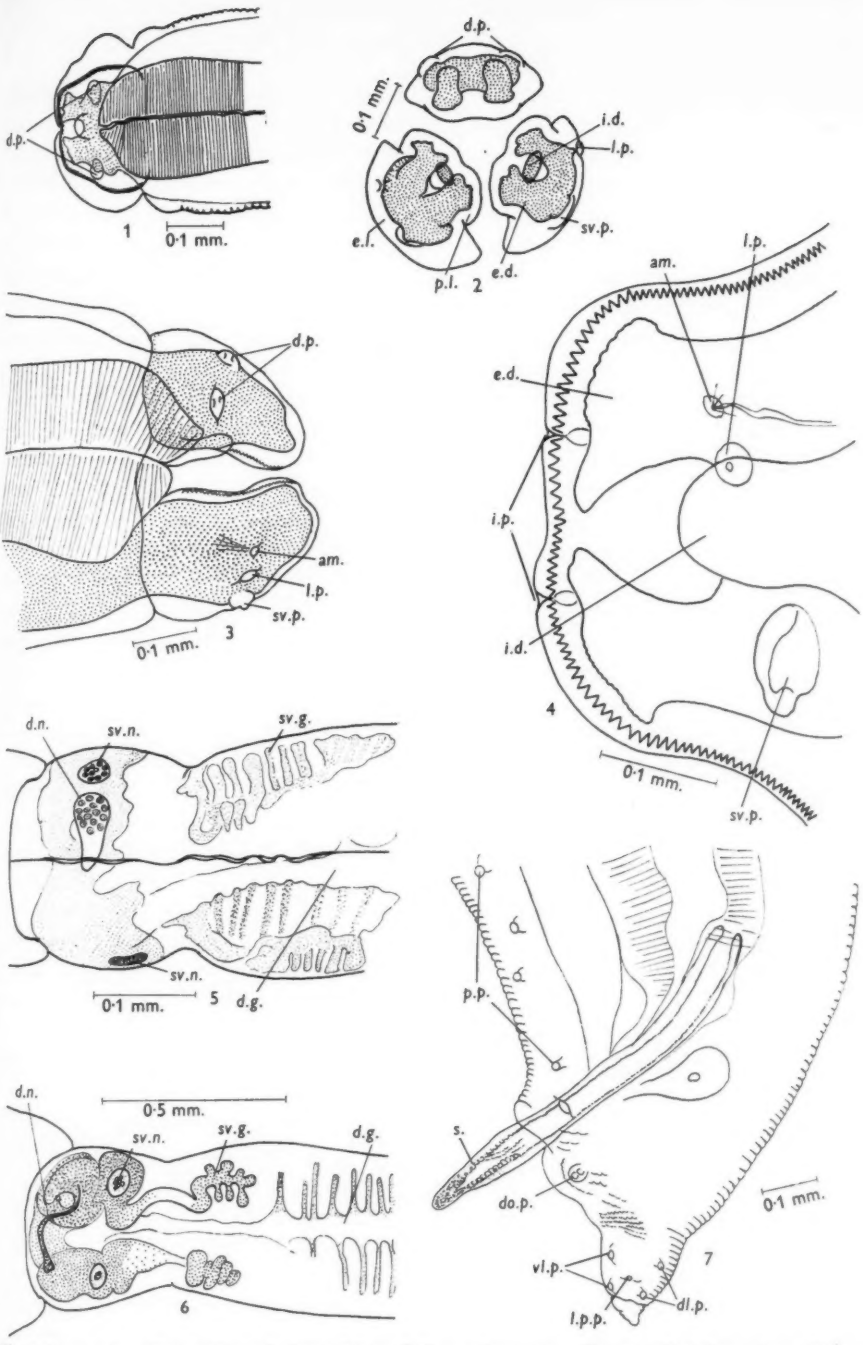
On 18 October 1955, Dr Josephine Mackerras, parasitologist at the Queensland Institute for Medical Research, sent to the writer a mature male and two mature female ascaridoids which she had collected from the small intestine of an Australian allied rat, *Rattus assimilis*.

I. DESCRIPTION OF *NEOASCARIS MACKERRASAE* N.SP.

Length of six living mature females 40–62 mm., length of three living mature males 35–55 mm. Pinkish white in colour, coils of gonads visible through cuticle. Measurements of the type specimens are shown in Table 1.

Table 1. *Measurements in mm. of the type specimens of* Neoascaris mackerrasae *n.sp.*

	Male	Female
Length	35	48
Width (at level of ventriculus)	0·662	0·960
Dorsal lip (length)	0·134	0·221
Dorsal lip (width)	0·192	0·288
Subventral lip (length)	0·158	0·101
Nerve ring (from anterior end)	0·547	0·826
Excretory pore (from anterior end)	0·624	0·893
Cervical papillae (from anterior end)	0·480	0·893
Oesophagus (length)	2·431	2·764
Oesophagus (width anterior end)	0·192	0·298
Oesophagus (width posterior end)	0·288	0·326
Ventriculus (length)	0·259	0·413
Ventriculus (width)	0·278	0·422
Vulva (distance from anterior end)	—	11·1
Anus (to tip of tail)	—	0·672
Phasmids (to tip of tail)	—	0·096
Spicules	0·547	—
Cloaca (to tip of tail)	0·264	—
Caudal region	0·125	—
Cuticular striations (width at level of ventriculus)	0·171–0·247	0·171–0·247



Text-figs. 1–7. Fig. 1. Dorsal view of lips of *N. mackerrasae*. Fig. 2. End face view of lips of *N. mackerrasae*. Fig. 3. Lateral view of lips of *N. mackerrasae*. Fig. 4. Subventral lip of *N. mackerrasae*. Fig. 5. Ventricular region of *N. mackerrasae*. Fig. 6. Ventricular region of *N. vitulorum*. Fig. 7. Lateral view of tail of male of *N. mackerrasae*.

Key to lettering, p. 360.

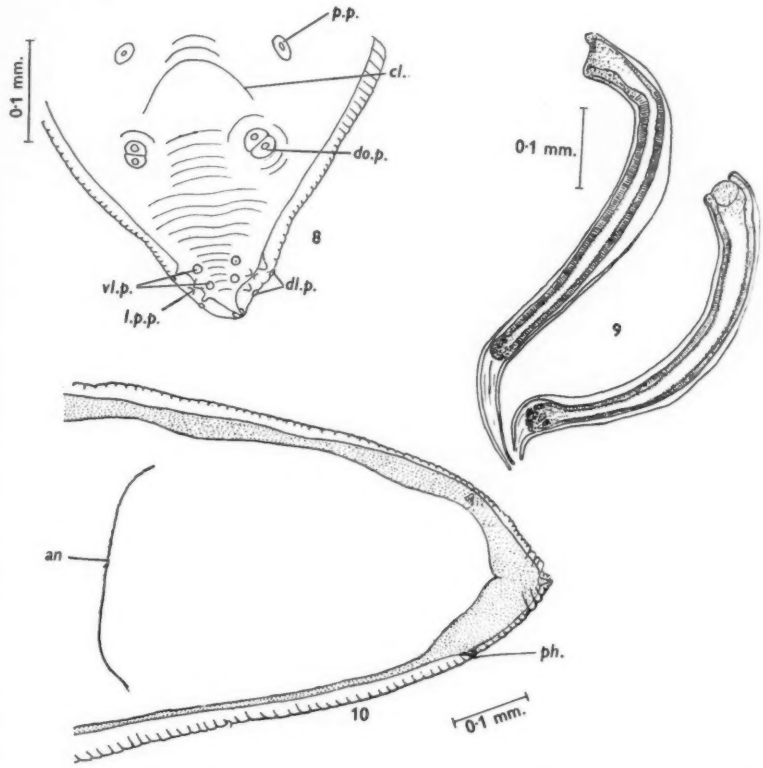
Three well-defined lips, one dorsal and two subventral, each demarcated from body by a deep groove, cuticle slightly swollen behind groove (Text-fig. 1). Interlabia absent. Each lip divided into oval posterior part (eulabium) and trapezoidal anterior part (prelabium) (Text-fig. 2); lip pulp does not fill eulabium, giving appearance of lateral cuticular expansions (labial wings); eulabium bears external ring of labial papillae (Text-fig. 2). External papillae consist of (1) two large oval papillae on dorsal lip (Text-figs. 1, 2); (2) one large oval ventro-lateral papilla on each subventral lip (Text-figs. 2, 3); (3) two lateral papillae on each subventral lip (Text-fig. 3). The more ventral of the two lateral papillae is circular and is less distinct and smaller than the large ventro-lateral papilla (Text-figs. 3, 4); the more dorsal member is elliptical, with conspicuous striations and a duct leading towards the eulabium, this papilla probably represents the amphid (Text-fig. 4). The prelabium contains three anteriorly directed prolongations of pulp, an unpaired internal prolongation and two external prolongations, the latter separated from each other by a deep cleft (Text-figs. 1, 2 and 4). Each external prolongation is cleft at the tip, divided into a smaller inwardly directed internal lobe and a larger external lobe with a rough edge (Text-fig. 4). In the depression thus formed on the anterior margin of each external prolongation is a minute vesicle communicating with a pit in the cuticle on the margin of the lip (Text-fig. 4); these structures probably represent six internal labial papillae. Dentigerous ridges situated just inside border of lip, denticles almost triangular in shape, average length 0·0037 mm., but those on the anterior border of the lip are larger (Pl. XVII, fig. 1).

Narrow cervical alae present, but only visible in transverse sections of oesophageal region, each wing is supported by a cuticular bar which is V-shaped in cross-section, the point being directed outwards (Pl. XVII, fig. 2). The alae are most conspicuous in the region anterior to the opening of the dorsal oesophageal gland, they disappear at about the middle of the oesophagus. Cervical papillae are inconspicuous, lying on each side in the region of the excretory pore.

Oesophagus divided by a constriction into anterior muscular section, containing follicles of dorsal and subventral oesophageal glands, and posterior ventricular portion (Pl. XVII, fig. 3), containing nuclei of dorsal and subventral oesophageal glands. Subventral glands extend forwards within oesophageal wall, but are restricted to its posterior part (Text-fig. 5), each opens by a fine duct into the oesophagus at a point slightly anterior to the junction of the oesophagus and ventriculus. The subventral glands are continued backwards in the ventriculus. In its anterior portion they are hardly visible, but in the central region they each enlarge into a glandular mass, more or less triangular in cross-section, which lies in the dorso-lateral field of each subventral sector of the ventriculus. Each triangular mass contains a spherical nucleus (Pl. XVII, fig. 4). The dorsal oesophageal gland appears to be considerably more developed than the subventral glands, it extends almost the whole length of the oesophagus and opens into the lumen by a minute duct at a point anterior to the nerve ring. The gland is reduced in the anterior part of the ventriculus, but in its posterior part it lies in three aggregations adjacent to the three radii of the lumen of the ventriculus and in its posterior part it forms a complete circle. The nucleus of the dorsal gland is oval and is situated very slightly

behind the other two nuclei, on the right side of the dorsal sector (Pl. XVII, fig. 4). Ventricular appendix and intestinal diverticulum absent.

The excretory system consists of two longitudinal vessels, that on the left is wider than the vessel on the right side in the anterior part of its course. The excretory nucleus is situated at the anterior end of the left vessel, and is relatively small and stains deeply.



Text-figs. 8–10. Fig. 8. Ventral view of tail of male of *N. mackerrasae*. Fig. 9. Spicules and spicule sheaths of *N. mackerrasae* (flanges not shown). Fig. 10. Ventral view of tail of female of *N. mackerrasae*.

Tail of male with 17–20 precloacal papillae arranged in two rows, no single unpaired papilla in front of cloaca. Cloacal opening borne on a distinct mound, bearing no rough areas (Text-fig. 7). Large double papilla on each side of mound behind cloaca (Text-fig. 8). Tail sharply constricted, forming conical extremity which bears five papillae on each side, one pair dorso-laterally, one pair ventro-laterally and an unpaired intermediate papilla (Text-figs. 7, 8). Spicules U-shaped in cross-section, having a central rod and two ventrally directed membranous flanges (Pl. XVII, fig. 6), slightly curved, tip bulbous and sculptured with coarse irregularities (Text-fig. 9). Testis coiled around intestine in anterior half of body, coils

extending to junction between oesophagus and ventriculus. About halfway along body, testis merges into vas deferens, then widens into capacious seminal vesicle which runs backwards and narrows into tubular portion which ends in a thick-walled spherical bulb. Ejaculatory duct with same width as bulb, but with thinner wall, narrowing gradually until it enters the cloaca.

In five living females the distance from the anterior end of the body to the vulva was 26–37 % of the total body length; vulva transverse, cuticle turning inwards to form lining of thin-walled vestibule whose lumen narrows into thick-walled vagina. In a female specimen 62 mm. long, the vagina extended backwards for 2 mm. and expanded into the undivided part of the uterus. The latter extended for about 4 mm. and divided into two thin-walled uterine tubes each running backwards side by side for a distance of about 14 mm. Posteriorly, they were adherent to each other for about 2 mm., but separated again as two narrow oviducts, each of which dilated into a fusiform swelling, narrowed again, and continued backwards for a short distance. One oviduct turned forwards and the coils of its ovarian filament extended to a point about 5 mm. in front of the vulva, the other oviduct continued backwards, its ovarian coils extending to a distance about 13 mm. from the posterior end of the body.

Female tail conical, bearing two lateral papillae (phasmids?) and a small button-like knob at the tip (Text-fig. 10). Eggs almost spherical and pitted with relatively large depressions (Pl. XVII, fig. 8), measuring 0·067–0·076 × 0·076–0·085 mm.

TYPE HOST: *Rattus assimilis* (small intestine).

TYPE LOCALITY: Subtropical rain forest at Mount Glorious, South Queensland, October 1955.

TYPE SPECIMENS: At the Queensland Museum, Brisbane; holotype (female) (G2390); allotype (male) (G2391); paratypes at the University of Queensland Veterinary School.

II. COMPARISON OF *NEOASCARIS MACKERRASAE* WITH *N. VITULORUM*

A comparison of specimens of *N. mackerrasae* with specimens of *N. vitulorum* showed that the two species resembled each other in the distribution and extent of the oesophageal glands (Text-figs. 5, 6), in the possession of an oesophageal ventriculus without appendix, though the ventriculus of *N. vitulorum* is less distinct, and in the presence of rudimentary cervical alae in the anterior cervical region; also in the morphology of the lips, the male and female reproductive organs (except the spicules) and the caudal region of the male. *N. mackerrasae* differed from *N. vitulorum* in the following features: (1) the nucleus of the dorsal oesophageal gland is oval and is situated in the dorsal sector of the oesophageal ventriculus (Pl. XVII, fig. 4; Text-fig. 5), whereas in *N. vitulorum* this nucleus is elongate and is situated in the subventral sectors (Pl. XVII, fig. 5; Text-fig. 6); (2) the vulva is situated posteriorly to the first quarter of the body, whereas it is situated in the anterior quarter of the body in *N. vitulorum*; (3) the eggs of *N. mackerrasae* are very coarsely pitted (Pl. XVII, fig. 8), whereas the eggs of *N. vitulorum* are more finely pitted; (4) the spicules are gutter-like, whereas in *N. vitulorum* they are cylindrical (Pl. XVII,

figs. 6, 7). Furthermore, the length measurements for *N. vitulorum*, cited by Baylis (1936*a*) as 85–150 mm. for the male and 155–300 mm. for the female, indicate that this species is much larger than *N. mackerrasae*.

III. REDEFINITION OF THE GENUS *NEOASCARIS*

The genus *Neoascaris* was proposed by Travassos (1927) because of the distinct ventriculus possessed by the bovine ascaris, whose morphology had been described by Neumann (1883), Ransom (1911), Baylis & Daubney (1922), Boulenger (1922), and Macfie (1922), and which had previously been known as *Ascaris vitulorum* Goeze, 1782. Travassos named *Neoascaris vitulorum* as the type species. This species was redescribed by Baylis (1936*a*), but not considering the presence of a ventriculus sufficient reason for the establishment of *Neoascaris*, he retained *Ascaris vitulorum*. Some doubt had been expressed by Macfie (1922) as to the uniformity of the specimens collected in different parts of the world, but Baylis regarded it as unlikely that more than one species could have been confused, and considered it more probable that the species is somewhat variable. Variations appear particularly in the number of papillae on the male tail. *Neoascaris* was revived by Chitwood (1937) and has since been generally accepted. The genus was defined by Mozgovoi (1953) and, as the definition was in Russian, a translation is given below:

> Three lips each with dentigerous ridges; pulp of each lip with 2 anterior lobes divided by a deep median groove, and a single median lobe. Interlabia absent. Cervical alae absent. Muscular oesophagus separated from intestine by a granular ventriculus. Male with no caudal alae: pre-anal papillae and post-anal papillae present; spicules short, almost equal and non-alate. Female with vulva in anterior half of the body; two-branched uterus; eggs with thick, wrinkled covering. Parasites of ruminants. Type species: *Neoascaris vitulorum* (Goeze, 1782), Travassos, 1927.

Up to the present time *N. vitulorum* has remained the only species in the genus, but as this is the only generic definition which fits the parasite described in this paper, *N. mackerrasae* constitutes the second species.

Mozgovoi included *Neoascaris* in the family Anisakidae, but this family has been emended by Hartwich (1954) and restricted to forms having a uniramous excretory system. Hartwich has proposed that ascaridoids possessing a biramous excretory system, and having an oesophagus with globoid or spherical pars posterior without appendix, and with lips possessing labial wings and semicircular or trapezoid prelabium, should be included in a separate family which he named Toxocaridae, based on *Toxocara* Stiles, 1905 (originally monotypic for *T. canis* Stiles, 1905). As these characters are possessed by *Neoascaris*, it is proposed that this genus should be transferred to the family Toxocaridae, and redefined as follows:

Dentigerous ridges prominent; prelabium trapezoid, eulabium with cuticular expansions; pulp of each lip with three anterior prolongations, one internal unpaired prolongation and one pair of external prolongations separated by deep cleft; external prolongations cleft at tip into smaller inwardly directed lobe and larger external lobe. Internal labial papillae in cleft at tip of external prolongations.

Interlabia absent. Cervical alae reduced to a short lateral ridge supported by a V-shaped bar. Oesophagus divided into anterior muscular part and posterior ventriculus without appendix. Dorsal oesophageal gland extends forwards beyond nerve ring, subventral eosophageal glands restricted to posterior region of oesophagus. Vulva in anterior half of body; uterus opisthodelphic, unpaired part less than half length of paired portion.

Tail of male with terminal conical appendage bearing two or more pairs of papillae on each side. In addition, a large double papillae on each side behind cloaca. Several pairs of pre-cloacal papillae arranged in two rows. Spicules cylindrical or gutter-like. Parasites of mammals.

TYPE SPECIES: *N. vitulorum* (Goeze, 1782).

Comparison of *N. vitulorum* and *N. mackerrasae* with two available species of *Toxocara*, namely *T. canis* and *T. cati*, showed that the cervical alae of *T. canis* though considerably more prominent are basically similar in structure to those possessed by *Neoascaris vitulorum* and *N. mackerrasae*. Furthermore, the spicules of *N. mackerrasae* closely resembled those of *Toxocara canis*, although in the latter species the gutter-like form of the spicules is exaggerated to the extent that they are described by Baylis (1936*a*) as 'alate'. The characters which differentiated both *Neoascaris* spp. from both the above-mentioned *Toxocara* spp. were (1) that the undivided part of the uterus in the former was less than half the length of the divided part of the uterus, whereas in the latter the undivided part was more than half as long as the divided part; (2) that the external prolongations of the labial pulp are subdivided at the tip in both *Neoascaris* spp., whereas in both *Toxocara* spp. they were undivided.

IV. OTHER ASCARIDOID PARASITES OF RODENTS

The first ascaridoid to be reported in rodents was *Ascaris castoris* Rudolphi, 1809. According to Rudolphi (1809, p. 195), who named but gave no description of this parasite, Perrault and Charras found eight specimens in the intestine of a beaver. This parasite has not been found again until recently, when it was collected and described from the beaver (*Castor fiber*) in Western Europe (Mozgovoi, 1953, vol. 1, p. 74). Leidy (1856) found a single female ascaris in a woodchuck (*Marmota monax*) and named it *Ascaris laevis*. Tiner (1951) provided a more detailed description of *A. laevis*, collected from *Marmota monax* in Pennsylvania and from *Citellus parryi barrowensis* in Alaska. Von Linstow (1897) described *Ascaris pigmenatata* from the marmot (*Marmota marmota*); this parasite had dark pigment occurring throughout the cuticle, walls of the intestine, and oesophagus; it possessed interlabia which in von Linstow's figure appear quite conspicuous. On this account the species has to be retained as distinct from *Ascaris laevis*. According to Hall (1916), Parona (1909) found a single female worm in the stomach of *Mus minutoides* from Africa, the parasite was referred to as '*Ascaris* sp.', but there was no description.

Schulz (1931) described *A. joffi* and *A. tarbagan* from the intestine of *Citellus pygmaeus* and *Marmota sibirica* respectively. The two species were very similar, the former had single papillae on the lips, whereas the latter had double papillae

and, apart from *Ascaris joffi* being smaller, no other differences were mentioned and no males of *A. joffi* were found. From Schulz's description of *A. tarbagan* and Tiner's description of *A. laevis*, it is evident that these two species, if not identical, are very closely related. The only difference appears to be that Schulz made no mention of any pericloacal rough areas which Tiner described in *A. laevis*. According to Tiner (1951), Linsdale (1946) reported *A. columnaris* from a ground squirrel (*Citellus beecheyi*), but Tiner (1951) re-examined these specimens and stated that they were more probably variants of *Ascaris laevis*.

Several authors have reported *A. lumbricoides* in rodents. Baylis & Daubney (1922) found worms identical, as regards the eggs, the lips and the male tail, with *A. lumbricoides* in squirrels (*Sciurus indicus*, *S. pygerythrus*) from India. Rausch & Tiner (1948) collected a male *Ascaris lumbricoides* from a fox-squirrel (*Sciurus niger*) in Wisconsin, as well as immature forms which they did not identify. Tiner (1951) examined three vials containing ascaridoids collected from fox-squirrels in the United States, and in two instances he considered that the worms were definitely *Ascaris lumbricoides*. Tiner & Chin (1948) and Tiner (1951) identified as *A. lumbricoides*, ascaridoids from the muskrat (*Ondatra zibethica*).

In addition to the description of *Ascaris castoris* after Moskalew, Mozgovoi (1953) gave descriptions of two species of ascaris collected in rodents in the U.S.S.R. He listed them as *A. spalacis* Schulz & Alojan, 1950, from the mole-rat (*Spalax leucodon*), and *Ascaris brevispiculum* Sadovskaya, 1952, from the field mouse (*Apodemus agrarius*), the original description of the latter as cited by Mozgovoi appears to be in manuscript.

A comparison of *Neoascaris mackerrasae* with these descriptions showed that it differed from *Ascaris laevis* in not possessing any precloacal rough areas and from *A. tarbagan* in possessing only half the number of pre-cloacal papillae. It also differed from both these species and from *A. joffi* in that the egg-shell was more coarsely pitted. In size, in the number of pre-cloacal papillae, and in the body measurements (Table 1) this parasite approached most nearly to *A. brevispiculum*. It differed from this species in that: (1) the spicules were more than twice as long, (2) the vulva was situated about one-third the way along the total length, (3) there was more than one papilla on each of the subventral lips, (4) there were five pairs of post-cloacal papillae, and (5) the egg-shell was coarsely pitted. *A. castoris*, *A. spalacis* and *A. lumbricoides* are all larger species, with relatively longer spicules, oval eggs, and a larger number of pre-cloacal papillae.

Besides its spherical, coarsely pitted eggs, the character which differentiated *Neoascaris mackerrasae* from the descriptions of all the above-mentioned species was the possession of an oesophageal ventriculus.

V. DISCUSSION

The significance of the oesophageal glands in the phylogeny of the Ascaridoidea was discussed by Hsü (1933). He demonstrated in *Toxocara canis* and *T. cati* that the dorsal gland extends the whole length of the oesophagus, opening into the oesophagus at a point anterior to the nerve ring, that the subventral glands are much shorter and are restricted to the posterior part of the oesophagus, and that the

nucleus of the dorsal gland lies within the ventral part of the ventriculus. He pointed out that in these respects these species differ from other species occurring in terrestrial mammals such as *Ascaris lumbricoides*, *Toxascaris leonina* and *Parascaris equorum*.

It is interesting to note that Hsü (1933) concluded that the primitive arrangement of the nuclei of the oesophageal glands probably consisted of three nuclei of equal size situated in the centre of each sector of the oesophagus. Furthermore, Hartwich (1954) suggested that the forms possessing a ventriculus are more primitive than those without. Hence *Neoascaris mackerrasae* appears to be the only species known which possesses a ventriculus containing three equal-sized and almost symmetrically placed nuclei. Hence, if the above hypotheses are correct, this species may represent a primitive form among ascaridoid species. The displacement of the nucleus of *N. vitulorum* into the ventral part of the ventriculus possibly represents an intermediate stage between the ventriculus of *N. mackerrasae* and that of *Toxocara canis* in which species the dorsal oesophageal gland has undergone more extensive development. The close relationship between *Neoascaris* and *Toxocara* is further indicated by the similarity of the spicules in *Neoascaris mackerrasae* and *Toxocara canis* and by the presence of rudimentary cervical alae in both species of *Neoascaris*. These alae are considerably reduced compared with *Toxocara canis*, but it appears from the description of *T. pteropodis* by Baylis (1936*b*) that the cervical alae in this species were almost as narrow as those of *Neoascaris mackerrasae*. Further evidence of the affinities of *Neoascaris* and *Toxocara* must, however, await a re-examination of all species of the latter genus, with particular reference to the structure of the lips, cervical alae and oesophageal glands.

VI. SUMMARY

Neoascaris mackerrasae from the small intestine of the Australian allied rat (*Rattus assimilis*) is described. It is compared with *Neoascaris vitulorum* and found to differ as follows:

(1) It is a considerably smaller species.

(2) The vulva is situated more posteriorly.

(3) The nucleus of the dorsal oesophageal gland is oval and situated in the dorsal sector of the ventriculus, whereas in *N. vitulorum* it is elongate and is situated in the subventral sectors.

(4) The eggs are more coarsely pitted.

(5) The spicules have two flanges which form a gutter on the ventral surface, whereas in *N. vitulorum* they are almost cylindrical.

The genus *Neoascaris* is redefined so as to include the presence of rudimentary cervical alae and the unequal distribution of the oesophageal glands. The genus is placed in the family Toxocaridae Hartwich, 1954.

Other ascaridoid parasites occurring in rodents are listed and differentiated from *N. mackerrasae*. It is suggested that on account of the arrangement in the ventriculus of the nuclei of the oesophageal glands, *N. mackerrasae* may represent a primitive species among the ascaridoid parasites of mammals.

In presenting this work the writer takes pleasure in acknowledging the valuable technical assistance of Mr John Mines and Mr S. C. Ellis. The photographs were taken by Mr E. Hollywood of the Photographic Department of the University of Queensland. Thanks are due to Mr K. H. Webber for his assistance in trapping rodents at Mount Glorious.

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KEY TO LETTERINGS OF TEXT-FIGURES

am.	amphid	*i.p.*	internal labial papillae
an.	anus	*l.p.*	lateral papilla of subventral lip
cl.	cloaca	*l.p.p.*	lateral post-cloacal papilla
d.g.	dorsal oesophageal gland	*p.l.*	prelabium
d.n.	nucleus of dorsal oesophageal gland	*p.p.*	pre-cloacal papillae
d.p.	external papillae of dorsal lip	*ph.*	phasmid
dl.p.	dorso-lateral post-cloacal papillae	*s.*	spicule
do.p.	double post-cloacal papilla	*sv.g.*	subventral oesophageal gland
e.d.	external labial digitation	*sv.n.*	nucleus of subventral oesophageal gland
e.l.	enlabium	*sv.p.*	subventral papillae of subventral lip
i.d.	internal labial digitation	*vl.p.*	ventro-lateral post-cloacal papillae

EXPLANATION OF PLATE XVII

Fig. 1. Denticles of *N. mackerrasae* (× 844, enlarged twice).
Fig. 2. Cervical alae of *N. mackerrasae* (× 225).
Fig. 3. Ventriculus of *N. mackerrasae* (× 56).
Fig. 4. Nuclei of oesophageal glands of *N. mackerrasae* (× 225).
Fig. 5. Nucleus of dorsal oesophageal glands of *N. vitulorum* (× 112).
Fig. 6. Section of spicules of *N. mackerrasae* (× 225).
Fig. 7. Section of spicules of *N. vitulorum* (× 225).
Fig. 8. Egg of *N. mackerrasae* (× 675).

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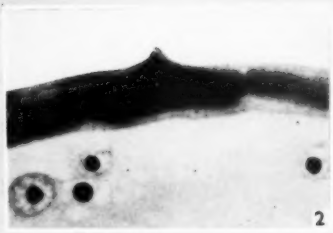

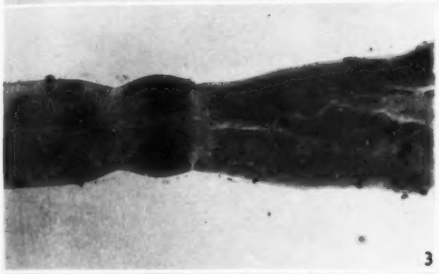

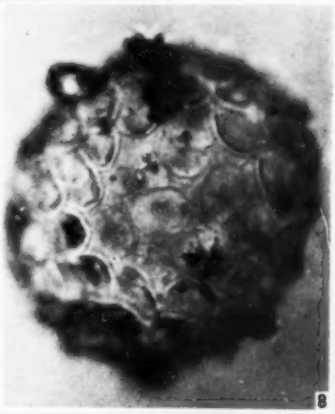

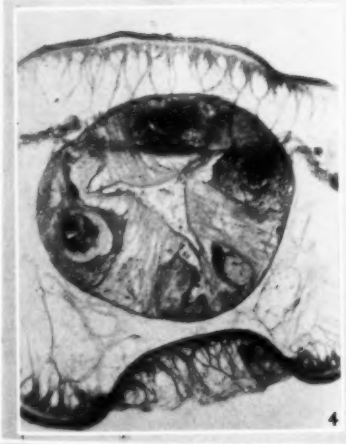

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OBSERVATIONS ON LABORATORY COLONIES OF THE TSETSE FLIES *GLOSSINA MORSITANS* WEST. AND *GLOSSINA AUSTENI* NEWSTEAD.

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I. INTRODUCTION

When working with tsetse flies it is often important that the vital statistics of the flies be known (e.g. age), but although these flies are maintained in a number of laboratories, the problem of maintaining a self-supporting colony without the addition of wild pupae is of long standing. The most notable success appears to have been with *Glossina palpalis* in West Africa (W.A.I.T.R. 1953, 1954, 1955), and there seems to have been little success with the East African savannah species. Willett (1953) reported his attempts with a number of these species, stating, however, that he did not undertake '...methods which could not be applied to large numbers, such as keeping flies singly in tubes'. This method was finally adopted in the present work as it gave better results, allowed records to be kept of individual flies, and thus gave much information which it was impossible to obtain when dealing with a cage-full of flies, and further, proved to be not an impractical method. With a small staff, of whom only one or two need be trained beyond ordinary reading and writing, it is possible to handle large numbers of flies under these conditions with much less time and trouble than would at first appear necessary.

The findings of Willett (1953) relate mainly to *G. swynnertoni*, and his figures for *G. morsitans* are derived from a total of only just over 100 flies. The present work has been concentrated on *G. morsitans*, and may be regarded as a sequal to Willett's work on the related species.

The observations recorded in this paper were not obtained under optimum conditions. In particular, lack of a continuous electricity supply led to difficulties in controlling temperatures and humidities; and, as the windows were sealed to minimize heat loss, the flies were illuminated for only 6–8 hr. per day. The use of controlled temperature and humidity cabinets is planned for future work, but as this will not be undertaken for some time, it is considered worth while to report the results so far obtained. Under more controlled conditions, results may well be improved.

The early attempts to maintain *G. morsitans* involved keeping the flies in boxes measuring $4\frac{1}{2} \times 3 \times 2\frac{1}{2}$ in. These were of wooden construction, but with the two larger sides composed of wire mesh of such a gauge as to retain the flies but to allow the larvae to pass through. Each box contained twelve flies (eight females and four males), and was laid wire-downwards in a separate tray containing dry sand to a depth of about 1 in.; gently pushing the box downwards into the sand enabled the flies to alight on the latter instead of the wire, and a card placed over half of the upper surface provided 'light' and 'shady' areas for the flies. Larvae passed through the wire mesh and pupated in the sand, which was searched weekly for pupae. Feeding was carried out by placing the boxes against the shaved skin of the feeding animal and holding them there for 15 min.; any flies intending to feed did so in that time.

A number of climatic conditions were tried, including those which proved optimum in later experiments, but in no case did the box-maintained flies live or breed satisfactorily. A major cause of death appeared to be an artificially produced exhaustion. The boxes quickly became impregnated with the smell of the feeding animal, and when the flies became hungry, this smell stimulated them to continual flying and probing. When food was eventually offered, many flies were too exhausted to feed.

The method was abandoned for reasons given previously, and later attempts at maintenance were based on the keeping of flies singly in tubes.

II. *GLOSSINA MORSITANS:* MAINTENANCE

The flies were maintained singly in numbered 3×1 in. specimen tubes. They spent most of the time resting quietly on the bottoms of the tubes. The bottom of each tube was covered on the inside by a circle of filter paper to absorb moisture and excreta, and each tube was closed by a piece of mosquito netting held in place by an elastic band. This netting was changed and washed frequently to prevent it becoming soiled or impregnated with the smell of the feeding animal and, when the tube itself became soiled, the fly was transferred to a clean one, which was similarly numbered. The tubes stood in racks in iron trays containing sand to a depth of about 3 in. This helped to prevent breakage of the tubes, and provided a humidity control mechanism; by moistening the sand with water it was possible to control the humidity of the air surrounding the flies, although not of the entire room.

Artificial light was available for 6–8 hr. per day, the illumination on the flies being approximately twenty foot-candles. Since the close of these observations,

flies have been maintained where daylight (about four foot-candles on the flies) has been available. Feeding has improved under these conditions, and it seems probable that a longer daily period of illumination would have improved the results from the colony.

A daily record was kept of each fly; hatching, feeding, mating, pupa production, obvious abortions and death were recorded.

Mating was carried out in the tubes, a male fly being transferred from its own tube to one containing a female fly. The flies were left together for 17–24 hr., and copulation occurred satisfactorily under these conditions. Best results were obtained by using males 10–14 days old and females 3–4 days old. Teneral flies were not mated, and all males were fed immediately before being introduced to the female. This prevented a number of post-coital deaths which at one time occurred among the males. Larvae were deposited in the tubes and pupated normally, the pupae being removed to wooden boxes containing sand to a depth of about 1 in. A hole 1 in. in diameter was cut in the lid of each pupa box, and the newly emerged flies, being attracted to the light, flew up through the hole into an upturned bottle. The pupae were subjected to the normal temperature variations of the room (see below) and to a humidity of 55–65% R.H. Hatching was satisfactory under these conditions, but raising the humidity to 80% R.H. considerably lowered the percentage emergence.

A number of temperature variations were tried, and the optimum proved to be a diurnal range of 74–80° F. During the mornings the temperature was raised to 79–80° F., feeding being carried out at this room temperature. The room was maintained like this for 3 or 4 hr., and then allowed to cool over the next 4 or 5 hr. to the night temperature of 74–75° F. The optimum atmospheric humidity for the flies was found to be 60–75% R.H.

The exact methods used in the feeding of the flies were most important. If the flies were offered food daily, a number of partial feeds were taken, and this had a deleterious effect on pupa production. To prevent this, and in view of the findings given in § III (iii), each fly was offered food every fourth day. Thus, a fly fed on Monday was offered food again on Friday; if it fed, its next meal was due on the following Tuesday. But if it did not feed on the Friday, it was offered food daily until it did feed, and the four-day system was restarted from that point. Newly emerged flies were offered food daily until they had taken three meals, and were not put on to the four-day system until these meals had been taken. These newly emerged flies, particularly of the second and third generations, often showed a disinclination to feed if the previous overnight temperature had dropped below 74° F. This is of interest because the minimum temperature in their natural habitat must frequently drop below this limit. Such temperatures in the colony, however, caused about 75% of the newly emerged flies to refuse food, and virtually all these flies died within 48–72 hr.; those which did feed appeared to live normally.

The flies due to feed on a particular day were arranged in their tubes, with as little disturbance as possible, in groups of ten. Each group was bound together with an elastic band, and placed netting-downwards on the skin of the feeding animal, feeding taking place through the netting. The tubes were held in position

for 10 min., during which time 50–60 % of the flies took full feeds; the group was then placed aside for 15–20 min., and then returned to the feeding site, when a further 20–30 % of the flies fed. Thus 70–90 % of the flies due to feed on that day were induced to do so.

The temperature at the feeding site proved to be critical. Feeding was carried out in the room, with the animal nearer to the heat source than the flies' normal habitat, the temperature at the feeding site being 80–81° F.; feeding at 79° F. was only fair, and it was quite inadequate at 78° and 82° F.

Although usually fed in the normal room illumination, at one time the only available light was afforded by two kerosene pressure lamps. By altering the position of these lamps it was found that the minimum light intensity required for feeding was 1·0–2·0 foot-candles at the feeding site.

The feeding animals throughout most of the period were sheep. From observations elsewhere, goats appeared to be acceptable, but figures for longevity and pupa production of goat-fed flies are not available. Originally rabbits and guinea-pigs were used, but a change to sheep resulted in a more satisfactory pupa production. However, in many cases where difficulty was encountered in persuading newly emerged flies to take their meals from sheep, a change to guinea-pig resulted in immediate feeding. This method was also employed for older flies which had refused to feed for some considerable time and were in imminent danger of dying of starvation. It was customary to select, daily, those flies which had not fed for 10 days or more, and to offer them guinea-pig; this often resulted in 50–60 % of these flies feeding, after which they were returned to the normal routine on sheep, where they fed satisfactorily.

Five feeding sites were used on each sheep; the flanks, the thighs, and the underside of the tail. Each area was shaved because any hair impeded feeding, and the areas were used in rotation. After feeding lanolin was thoroughly massaged into the skin, which was washed with warm soapy water before being used again. Even so, after some time the skin became too calloused for the flies to pierce, and new sheep were required every 4–6 weeks; resting the animal for a period was unsatisfactory because the skin, although it regained its suppleness, usually hardened again within a few days of the recommencement of feeding. It was noticed that even before the skin hardened, a change to a new animal resulted in better feeding, and this may indicate some physiological condition. Where large numbers of flies are fed, it is recommended that the feeding animal be changed frequently.

III. *GLOSSINA MORSITANS:* COLONY DATA

The colony originated from a batch of wild caught pupae, and three principal facts emerged from the subsequent history. First, the number of flies increased satisfactorily for a time, a steady and progressive rise in total numbers of about 7 % per week being observed. But secondly, each generation appeared less virile than the previous one, and thirdly, only while the first generation was the predominant part of the colony did the latter show an increase in total numbers. Later a steady decline was observed.

(i) *Longevity*

Data for longevity are given in Tables 1 and 2.

Table 1. Glossina morsitans: *Longevity*

	No. of flies		Mean life (days)	
	Male	Female	Male	Female
First generation: all flies	259	280	*41·0*	*60·8*
First generation: flies living more than 10 days	194	225	52·5	74·1
Second generation: all flies	124	143	*48·9*	*61·9*
Second generation: flies living more than 10 days	92	106	64·4	81·9
Third generation: all flies	29	39	*27·5*	*24·6*
Third generation: flies living more than 10 days	20	19	38·2	47·0

In the first generation the longest lived fly survived for 242 days, in the second generation for 179 days, and in the third generation for 103 days. These lives were all attained by female flies.

It is well known that female flies live considerably longer than the males under natural conditions. This is usually explained as being due to the males' greater activity and their suffering heavier losses by enemies (Jackson, 1937, 1944). In the colony, however, despite the absence of enemies and an apparent equality of activity, the females still consistently lived about a 30% longer time than the males. This value corresponds closely to the figure of 25% given by Potts (1940). The earlier deaths of the colony males appeared to be correlated with feeding difficulties. Throughout the observations the females usually fed normally up to the time they died (presumably) from old age, whereas many males died after refusing food for periods of 10–20 days.

Table 2. Glossina morsitans: *Rate of death*

	% dead before reaching age of					
	11 days		21 days		31 days	
	Male	Female	Male	Female	Male	Female
First generation	25·1	19·7	55·6	51·1	63·3	58·6
Second generation	25·8	25·9	31·5	31·5	40·3	39·2
Third generation	31·1	51·3	51·7	59·0	65·5	66·7

Although the difficulty of persuading newly emerged flies to feed was overcome in many cases, it nevertheless led to a high mortality among those young flies, as is shown in Table 2. In the first and second generations about one-quarter of the flies died during the first 10 days of life, and in the third generation a half of the females died during this period. After the first 10–20 days there was a distinct drop in the death rate, and the colony would have been more successful if the high initial mortality could have been curbed.

(ii) *Mating*

Spermathecal examination of flies from two smaller colonies maintained similarly to the main one, showed that all the first generation and 91 % of the second generation females were fully inseminated. These figures were confirmed by examination of dead females from the main colony. Dissections of third generation flies were too few to provide reliable results. Data from the main colony are given in Table 3. From the established formula (East African Tsetse and Trypanosomiasis Research and Reclamation Organization 1955), 25 days appeared to be a reasonable estimate for the first larval period, and the figures in the table are derived only from those flies which lived for more than 25 days; flies living less than this period could not, even if mated, reasonably be expected to produce pupae, although two first generation and one second generation females did in fact do so. These flies are not included in Table 3.

Table 3. Glossina morsitans: *Mating (flies living more than 25 days)*

	No. of flies mated	No. producing pupae	% producing pupae
First generation	115	73	*63·5*
Second generation	94	36	*38·3*
Third generation	14	2	*14·3*

It is seen that the proportion of mated flies producing pupae was reduced from each generation to the next. As at least the first and second generations were showing 90–100 % successful insemination, the reduction must be ascribed to a general lessening of virility of the flies.

(iii) *Pupa production*

In this paper the word 'pupa' refers only to a normal sized pupa, presumably viable at the time of formation. Small, ill-formed pupae, or abortions, are not included.

Among the first generation flies the maximum number of pupae produced by one female was ten, among the second generation eight, and from the third generation one fly produced one pupa and another produced three. Table 4 gives the distribution of fecundity in each generation.

Table 4. Glossina morsitans: *Distribution of fecundity*

	No. of flies producing No. of pupae									
	1	2	3	4	5	6	7	8	9	10
First generation	9	12	12	4	7	9	10	6	3	3
Second generation	13	5	6	8	4	—	—	1	—	—
Third generation	1	—	1	—	—	—	—	—	—	—

The frequency distribution of fecundity appeared to be bimodal, with the first peak among those flies which produced one to three pupae, separated by a trough from the second peak among those flies which produced more. The first peak was

due to at least two causes. First, a number of young flies died after producing one or two larvae, and secondly, some flies suffered a number of abortions before depositing normal larvae towards the ends of their lives. In one case the first larva was produced 105 days after mating. Frequently, after the first larva had been finally deposited, others followed at normal intervals, but only one or two such larvae were laid before the flies died of old age.

The most frequent interlarval period was 13 days, but the following periods were also unusually common: 24–25 days, 39–40 days, 52 days, and 78 days. These figures are all approximate multiples of 13.

Table 5 shows the mean number of pupae for all females in the colony, and Table 6 takes account only of those flies which were mated and lived for more than 25 days.

The mean pupa production of such females is given in column A, and column B shows the mean pupa production of those flies which actually produced pupae (referred to as 'producing females'). In all cases the values decreased from each generation to the next.

Table 5. Glossina morsitans: *Mean pupa production* (1) (*all flies*)

	No. of female flies	No. of pupae	Mean no. of pupae per female
First generation	280	349	*1·25*
Second generation	145	101	*0·71*
Third generation	39	4	*0·10*

Table 6. Glossina morsitans: *Mean pupa production* (2) (*mated flies living over 25 days*)

	No. of pupae	No. of mated females living more than 25 days	Mean no. of pupae per such female (A)	No. of females producing pupae	Mean no. of pupae per producing female (B)
First generation	349	115	*3·00*	75	*4·65*
Second generation	101	94	*1·10*	37	*2·73*
Third generation	4	14	*0·29*	2	*2·00*

The timing of the feeds taken by a female fly affected the duration of the interlarval periods. In the data presented here the first larval period has been omitted, the figures dating from the first truly interlarval period.

The average interval between feeds during an interlarval period did not affect the length of such a period. No correlation was found in approximately 350 interlarval periods ranging from 9 to 78 days duration. The average interval without food varied from 2·57 days (interlarval period 18 days) to 5·53 days (interlarval period 20 days).

Pupae were collected from the tubes each morning before the flies were fed. Some females which had deposited larvae fed later on the same day, but this had no significant effect on the length of the new interlarval period. Data are given in Table 7.

Table 7. Glossina morsitans: *Effect of feeding on day of parturition*

	Flies feeding on day of parturition		Flies not feeding on day of parturition	
	No. of cases	Mean interlarval period (days)	No. of cases	Mean interlarval period (days)
Colony 1: First generation	145	*15·43*	122	*16·88*
Second generation	35	*16·71*	29	*19·66*
Third generation	(1)	(20·00)	(1)	(13·00)
Colony 2: First generation	85	*18·81*	103	*18·87*
Colony 3: First generation	20	*12·45*	32	*16·13*
Total	286	*16·42*	287	*17·85*

Although feeding on the day of parturition appeared to decrease the following interlarval period by about 8%, this difference is statistically insignificant.

It was found, however, that the duration of an interlarval period was correlated with the maximum interval without food during the period, and that a number of feeds taken at short intervals were unable to counteract the effect of a single longer interval without food. It was immaterial at what stage of the interlarval period the maximum foodless interval occurred.

Table 8 gives the combined data from all colonies, and Table 9 the individual figures from each colony. The correlation is evident in all cases.

Table 8. Glossina morsitans: *Effect of maximum interval without food* (1)

Maximum interval without food (days)	No. of cases	Mean interlarval period (days)
3	54	*12·04*
4	213	*14·26*
5	151	*16·09*
6	64	*20·25*
7	52	*21·83*
8	22	*26·36*
9	9	*26·56*
10	6	*35·67*
11	4	*46·75*
12	(2)	(37·50)
13	(1)	(30·00)

A batch of newly emerged flies was set up as a controlled experiment to confirm these data, but extraneous conditions killed most of these flies before any results were obtained. Thus, the present figures must be regarded only as an interesting pointer. No effect was suspected until the data were analysed after the termination of the colonies, and these figures are presented in the belief that the idea will be substantiated when confirmatory experiments are possible.

In this paper the word 'feed' denotes a full feed. It is apparent that pupa production can be hastened by reducing the maximum interval without food;

reducing the interval between feeds to less than 3 days, however, led to the taking of partial feeds, which itself had an adverse effect on pupa production. Feeding every fourth day was adopted as the optimum.

Table 9. Glossina morsitans: *Effect of maximum interval without food* (2)

Maximum interval without food (days)	Colony 1						Colony 2		Colony 3	
	First generation		Second generation		Third generation		First generation		First generation	
	No. of cases	Mean interlaval period (days)	No. of cases	Mean interlarval period (days)	No. of cases	Mean interlarval period (days)	No. of cases	Mean interlarval period (days)	No. of cases	Mean interlarval period (days)
3	25	*11·72*	11	*13·36*	—	—	14	*11·36*	4	*12·75*
4	111	*14·84*	23	*14·70*	(1)	(13·00)	60	*13·20*	18	*13·78*
5	77	*16·62*	14	*17·86*	—	—	42	*15·69*	18	*14·89*
6	28	*18·82*	10	*24·70*	—	—	20	*21·85*	6	*14·17*
7	21	*19·14*	5	*27·00*	—	—	23	*23·35*	3	*20·33*
8	5	*22·40*	—	—	—	—	16	*28·50*	(1)	(12·00)
9	(1)	(56·00)	(2)	(33·50)	(1)	(20·00)	(3)	(22·33)	(2)	(14·50)
10	—	—	—	—	—	—	5	*37·60*	(1)	(26·00)
11	—	—	—	—	—	—	4	*46·75*	—	—
12	—	—	—	—	—	—	(2)	(37·50)	—	—
13	(1)	(30·00)	—	—	—	—	—	—	—	—

(iv) *Viability of pupae*

Of the original batch of wild-caught pupae, 63·3% hatched to give normal healthy flies. Of the pupae from these first generation flies 75·4% hatched successfully, and for the pupae from the second generation flies the figure was 67·5%. No hatches were obtained from the four pupae from the third generation flies. Thus, excluding the latter, there was no particular difference in the hatching of pupae from different generations.

(v) *Pupal weight*

Table 10 shows the pupal weight decline through the generations. The wild pupae were later determined (from emergence dates) to have a mean age of two

Table 10. Glossina morsitans: *Pupal weight*

Source of pupae	Mean weight (mg.)
Wild flies	23·7
First generation flies	21·6
Second generation flies	19·1
Third generation flies	19·2

to three weeks; weights given for other generations were determined on pupae one day old. Three interesting points arise from the table. First, there was a decrease in pupal weight from most generations to the next. But secondly, there was no weight decrease between pupae from the second and third generation flies. Although the third generation pupae were only four in number, the fact is possibly worthy of note, because the figures of Willett (1953) show a similar halt in the weight decline between his third and fourth generations. As Willett started with a

generation of wild-caught flies, his third and fourth generations would be roughly equivalent to the present second and third generations. Thirdly, although collected during the wet season the present wild pupae (at 2–3 weeks of age) showed a mean weight of only 23·7 mg., the figure declining to 19·2 mg. in the third generation. Willett's pupae from wild flies showed a mean weight of 30·70 mg., decreasing to 26·48 mg. in the equivalent time.

IV. THE *GLOSSINA AUSTENI* COLONY

(i) *Conditions*

A colony of *G. austeni* was maintained by a technique similar to that described for *G. morsitans*. Unfortunately no suitable environment was available and the flies were maintained for a long time in the *G. morsitans* room. These conditions were observably unsuitable, especially for the pupae, and the colony was eventually moved to a room with a diurnal temperature range of 80–85° F. and a humidity range of 77–82% R.H. This change led at first to an improvement in the colony, but later on light was restricted to only 30 min. per day. This resulted in an increased mortality, and eventually a complete cessation of pupa production. Thus, the data given here originate from flies which were continually subjected to unsuitable climatic conditions; nevertheless, the results are sufficiently striking to merit attention.

(ii) *Feeding*

Feeding was carried out as described for *G. morsitans*. Guinea-pigs and sheep appeared to be equally acceptable. Feeding was more satisfactory than among the *G. morsitans*, as is shown in Table 11.

Table 11. Glossina morsitans *and* G. austeni: *feeding*

Maximum interval without food (days)	% of interlarval periods involved	
	G. morsitans	*G. austeni*
3	9·34	45·95
4	36·85	43·24
5	26·12	6·08
6	11·07	4·73
7	9·00	—
8	3·81	—
9	1·56	—
10	1·04	—
11	0·69	—
12	0·35	—
13	0·17	—

Among the *G. austeni* no interlarval period involved an interval without food of more than 6 days. About 90% of the *G. austeni* interlarval periods involved a maximum foodless interval of not more than 4 days, compared with a figure of approximately 45% for the *G. morsitans*.

A number of newly emerged male flies steadfastly refused to feed and this was partly responsible for the high death rate among the males.

(iii) *Longevity*

The figures given in Table 12 for the male flies are somewhat unreal, because these flies withstood environmental changes less well than did the females, and each change was followed by a high mortality among the males. The figures for the females, however, show that in longevity the *austeni* flies surpassed the *morsitans* (Table 1).

Table 12. Glossina austeni: *Longevity of flies*

	No. of flies		Mean life (days)	
	Male	Female	Male	Female
First generation	100	62	*35·04*	*96·90*
Second generation	37	40	*19·94*	*46·68*
Third generation	6	4	*14·00*	*71·75*

(iv) *Pupa production*

Table 13 shows the pupa production within the colony. A comparison of these data with those given in Tables 5 and 6 shows the superiority of the *austeni* over the *morsitans* flies, despite the fact that they ceased to produce pupae when necessarily maintained in darkness. Although the later parts of their lives were barren, several of the first generation flies produced thirteen pupae, and several of the second generation produced nine.

Table 13. Glossina austeni: *Pupa production*

	No. of pupae	Total no. of females	Mean no. pupae per female	No. of producing females	Mean no. of pupae per producing female
First generation	194	62	*3·13*	27	*7·19*
Second generation	48	40	*1·20*	9	*5·33*
Third generation	(1)	4	(0·25)	1	(1·00)

The greater success in feeding the *austeni* flies makes it difficult to say whether the maximum interval without food during an interlarval period affects the duration of such a period in the same manner as in *morsitans*. For a maximum interval without food of 3 days the mean interlarval period was 10·94 days, for an interval of 4 days it was 13·67 days, for 5 days it was 13·11 days, and for 6 days it was 18·86 days. The latter two values, however, are based on only nine and seven cases, respectively.

The mean length of all *austeni* interlarval periods was 12·33 days (minimum 7 days) compared with 17·08 days (minimum 9 days) for the *morsitans*. This may be due to an intrinsic difference in the physiology of the two species, although the difference would appear to be rather large for this explanation. It is considered more likely to be a result of the more regular feeding of the *austeni* flies, leading to fewer abortions.

V. DISCUSSION

The results presented in this paper would undoubtedly have been improved by more controlled conditions. In particular the figures for the *austeni* colony were obtained under very inadequate conditions, and the comparative success of these flies

supports the suggestion of Willett (1953) that this species may become a valuable laboratory vector of trypanosomiasis. The ideal conditions for the pupae were not determined, with the result that very few pupae hatched, and this led to a drastic reduction in numbers from one generation to the next. If the pupal environment had been ideal, the overall picture of the colony would have been greatly altered.

In the *morsitans* colony, the early conditions were largely experimental, and the first generation flies suffered consequently both in longevity and pupa production. Nevertheless, a number of important points have been established.

Buxton (1955) repeatedly quotes work stressing the importance of satisfactory feeding. Mellanby (1937) showed that larvae of normal size could be produced in captivity if the mother fed sufficiently well, and noted that, as in the present work, partial feeds led to smaller larvae and more frequent abortions. The colonies described here suffered principally from a decrease in virility from each generation to the next, this being reflected in a corresponding decrease in pupal weight. If this decreased virility could be avoided, one could reasonably expect the pupa production to maintain a steady level through many generations, and this alone would be a major step forward in the problem of maintaining a breeding colony. The present work has shown a way in which not only the physiological condition of the larva, but also the number of larvae produced, is a function of maternal feeding. Buxton (1955) observed that larvae are deposited at regular intervals provided that the mother is able to obtain 'adequate nourishment'; it now seems probable that regularity of feeding (as distinct from the total intake of food) is the prime object to be attained, the interval between feeds to be as short as is consistent with the taking of full feeds.

If the length of the interlarval period is correlated with the maximum interval without food during the period, then it cannot be calculated with certainty from the well-known formula given by the East African Tsetse Organization (1955), which allows temperature as the only variable. If a similar state of affairs occurs in nature, it will be of great importance in relation to population studies; if, however, a wild fly can contact a host and feed whenever it wishes and is not compelled to wait until food is offered, then the effect may be purely a laboratory one.

Data are too few to allow any definite construction to be placed on the fact that 13 days and multiples thereof were more than usually common in the frequency distribution of fecundity, although it may indicate that abortions tended to occur at the ends of the interlarval periods.

Buxton (1955) notes the lower pupa production figures for captive flies compared with wild flies. In the present colonies, although those flies which produced pupae did so satisfactorily, the mean number of pupae for all females was low. This was partly because many of the flies died before they were old enough to produce larvae, and inadequate feeding was shown to be a cause of this. It is evidently important to devise some better method of feeding these young flies in order that they may survive to reproduce.

Temperature has been shown to be a critical factor in determining the success of feeding. In view of the importance of correct feeding, the principal object with a colony should be to regulate the temperature (and possibly other climatic

conditions) in order to obtain the most effective feeding conditions throughout life. If this can be done, then longevity, pupa production, and pupal weights are likely to show substantial improvements.

Even among those females reaching reproductive age, however, a proportion showed a marked tendency to abort. This has been noted by previous workers (various authors quoted by Buxton (1955)), but the reason is obscure. In the present work many of these flies produced normal larvae and then entered a phase of continual abortions, or vice-versa. Feeding was not observably worse during the abortive periods, and this problem too must be solved before a successful breeding colony can be maintained without the risk of periodic depressions in numbers.

VI. SUMMARY

(*a*) Glossina morsitans

1. A method of maintaining a breeding colony is described. The flies were best kept singly in tubes.

2. The optimum temperature and humidity ranges were 74–80° F. and 60–75 % R.H., respectively. Young flies failed to tolerate a lower minimum temperature.

3. A light intensity of at least one foot-candle was needed at the feeding site.

4. The method of feeding the flies was of prime importance. Best results were obtained by offering food every fourth day, at a temperature of 80–81° F. The best feeding animal was sheep, but guinea-pig was useful for inducing newly emerged flies to feed. The condition of the skin at the feeding site was important, and it is suggested that frequently changing the feeding animal will lead to better feeding.

5. Female flies lived longer than male flies, and in both sexes the highest mortality was during the first 20 days of life.

6. The optimum age for mating was 10–14 days for males and 3–4 days for females; 90–100 % of the first and second generation females were fully inseminated.

7. Each generation was less virile than the preceding one. Pupa production was decreased and the abortion rate increased from each generation to the next. Pupal viability, however, remained constant throughout all generations but the mean pupal weight showed a progressive decrease, except for the pupae from the second and third generation flies.

8. The mean interval without food during an interlarval period did not affect the duration of the period.

9. Feeding on the day of parturition did not significantly affect the length of the succeeding interlarval period.

10. The duration of an interlarval period was directly correlated with the length of the maximum interval without food during the period.

(*b*) Glossina austeni

11. A colony was maintained in a manner similar to that used for *G. morsitans*, but best results were obtained at 80–85° F. and 77–82 % R.H.

12. Feeding was more satisfactory than in *G. morsitans*.

13. In longevity and reproduction the flies were superior to *G. morsitans*.

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IMMUNITY IN TRYPANOSOMIASIS

I. NEUTRALIZATION REACTION

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(With 1 Figure in the Text)

Trypanosomiasis came into the field of immunological studies at the beginning of this century, when Laveran & Mesnil (1902) demonstrated the presence of protective antibodies in the serum of sheep and goats infected with *Trypanosoma brucei*. Their observations were confirmed by Martini (1905), Mesnil & Brimont (1909) and Kudicke (1911). After these promising early results, further studies on immunity in African trypanosomiasis presented some difficulties and produced controversial results. It was soon found that trypanosomiasis constituted immunologically a more complicated problem than bacterial infections at that date (Ritz, 1914). Since then the literature on immunity in Trypanosomiasis has been very scanty. Most of the published work consists of either field observations lacking fundamental studies, or laboratory studies of experimental animals cured with drugs and based on crude immunological tests. In evaluating any of the experiments dealing with immunity in trypanosomiasis it was noted that one of the serious handicaps to the studies of this problem was the lack of a method for the detection of antibody.

The present paper will describe the methods by which neutralizing or protective antibody can be demonstrated in the serum of animals either infected or hyperimmunized with dead trypanosomes, and the results of experiments designed to study various immunological problems in trypanosomiasis.

MATERIALS AND METHODS

Strains

The following strains were used in the experiment:

Trypanosoma brucei 'Shinyanga III', obtained from East African Central Trypanosomiasis Research Laboratory, Tororo, Uganda.

T. brucei '1932', obtained from Liverpool School of Tropical Medicine.

T. gambiense 'H', obtained from Liverpool School of Tropical Medicine.

T. rhodesiense 'L', obtained from Liverpool School of Tropical Medicine.

T. rhodesiense 'B', obtained from Bechuanaland through London School of Hygiene and Tropical Medicine.

T. congolense 'Vom', obtained from Liverpool School of Tropical Medicine.

All the strains were maintained in mice by syringe passage.

Consideration was given to the idea that it is essential in all work on immunity in Trypanosomiasis to keep the original passage strain of trypanosomes and any subsequent strain to be tested continuously in mice, which are quickly killed by

the parasite before antibodies develop. If strains are maintained in guinea-pigs or rabbits they become biologically changed, because of their constant contact with antibodies.

Preparation of suspension of trypanosomes for the test

Normal mice of approximately 20 g. weight were infected with the trypanosomes and bled at the peak of infection into modified Alsever's solution containing 10 % horse serum which will be referred to hereafter as 'A.S.' fluid. The mixture was left at room temperature for a period of about 1 hr., during which time the mouse erythrocytes were agglutinated by the horse serum and sedimented. Trypanosomes present in the supernatant fluid were transferred to another tube and centrifuged. Sedimented trypanosomes were washed with Alsever's solution and diluted with it to the desired concentration. The trypanosomes were then examined for vitality and were used within 4 hr. for the test.

Sera

Sera for the present experiment came from four groups of rabbits and two groups of other experimental animals.

The first group represented six rabbits injected intravenously with 1 ml. of *T. brucei* 'O' suspension containing 100,000 organisms per ml. Three of the animals in the first group were treated with one dose of 2 ml. of 1 % Suramin (Bayer 205) at the fourth week of infection.

The second group consisted of eight rabbits infected with other species of trypanosomes, such as *T. rhodesiense*, *T. gambiense* and *T. congolense*.

The third group of animals consisted of six rabbits hyperimmunized with *T. brucei* 'O' suspension killed by 0·5 % formalin. Rabbits were inoculated with 1·0 ml. of the suspension containing two millions of trypanosomes per ml. Injections were made every third day intravenously. A total of six injections was given.

The fourth group of animals represented rabbits infected with *T. brucei* 'O' which was passaged through rabbits. Sera of these rabbits were collected at weekly intervals.

The fifth group of animals consisted of guinea-pigs, rats and mice infected with *T. brucei* 'O'.

The sixth group of control animals represented normal healthy rabbits, guinea-pigs, rats and mice.

None of the sera used in the experiment was heated above 37° C. before the test and all the sera were stored at −40° C. until ready to be used.

EXPERIMENTAL

Neutralization test. The term neutralization test is used to refer to the mixture of organisms and antibody *in vitro*, with determination of inactivation of the organisms by inoculation into mice. Serial two-fold dilutions of the tested sera and normal control sera were made in Alsever's solution containing 10 % horse

serum (A.S. fluid) in 0·5 ml. volumes. Then to each tube 0·5 ml. of living trypanosomes suspended in A.S. fluid was added. The trypanosome-serum mixture was thoroughly shaken and left on the bench at approximately +20° C. for 1 hr.

It was found in the preliminary studies that the suspension of trypanosomes showing one or two trypanosomes per microscopical field after adding to diluted serum produced more constant results than other concentrations. It was also found that 1 hr. incubation at room temperature provided the necessary time for inactivation of the trypanosomes. After 1 hr. incubation the mixture was inoculated intraperitoneally into mice in a volume of 0·25 ml. for each mouse. Each dilution of the serum tested and of the control normal serum was inoculated into four mice. All inoculated mice were examined daily for the presence of trypanosomes in the blood by obtaining a drop of blood from the tail vein. Observations were carried out for a period of 21 days, although controls usually died of trypanosomiasis within 7 days. The results with sera collected after 4 weeks of infection in rabbits are summarized in Table 1.

Table 1. *Showing neutralization reaction with sera of infected rabbits* (T. Brucei 'O')

Dilution of serum	Mortality ratio of mice inoculated with mixture of serum and trypanosomes. Number of rabbit sera													
	Pre-infection						4 weeks after infection						Controls	
	1	2	3	4	5	6	1	2	3	4	5	6	C1	C2
1/10	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	1/4*	0/4	0/4	0/4	2/2	2/2
1/20	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	1/4*	2/4*	2/2	2/2
1/40	–	–	–	–	–	–	0/4	1/4*	0/4	0/4	0/4	0/4	2/2	2/2
1/80	–	–	–	–	–	–	0/4	0/4	0/4	0/4	0/4	1/4*	2/2	2/2
1/160	–	–	–	–	–	–	0/4	2/4	1/4	0/4	2/4	3/4	2/2	2/2
1/320	–	–	–	–	–	–	0/4	2/4	1/4	0/4	2/4	3/4	2/2	2/2
1/640	–	–	–	–	–	–	4/4	4/4	4/4	4/4	4/4	4/4	2/2	2/2

* Accidental death. –, Not tested.

Table 1 shows that rabbits infected with *T. brucei* 'O' produced neutralizing antibody which is able to inactivate completely trypanosomes in dilutions as high as 1/80 and in two cases in 1/320, while sera of these rabbits prior to infection like those of the control normal rabbits failed to show any neutralization even in dilution 1/10.

Development of neutralizing antibody in rabbits experimentally infected with T. brucei. In order to observe the development of neutralizing antibody in infected animals four rabbits were inoculated intravenously with a suspension of living *T. brucei* 'O'. These rabbits were twice bled prior to infection and, after infection, at intervals of 3 days in the first week of infection and thereafter once a week. After 4 weeks of infection two of the rabbits were treated with 2 ml. of 1% suramin intravenously and continued to be bled at weekly intervals in order to find the length of time neutralizing antibodies persist in the sera of these rabbits. The results of this experiment are shown in Fig. 1.

It has been demonstrated that neutralizing antibody in infected rabbits appeared 5 days after infection and gradually increased to a peak within 28 days. If these rabbits were treated 28 days after infection the maximum titre persisted for 98 days and then gradually decreased and disappeared after 22 weeks.

Development of neutralizing antibody in rabbits following several immunizing injections of dead trypanosomes. The results of early attempts to immunize laboratory animals with dead trypanosomes or their disintegration products were either almost entirely negative (Manteufel, 1909; Laveran, 1911) or simply prolonged the life of the immunized animal a short time over that of the control (Ross & Thomson, 1910; Kligler, Olitzki & Kligler, 1940) and it has been accepted generally that in trypanosomiasis immunity can be produced only during infection. Because of these conflicting results it was decided to reinvestigate the immunity produced by dead trypanosomes.

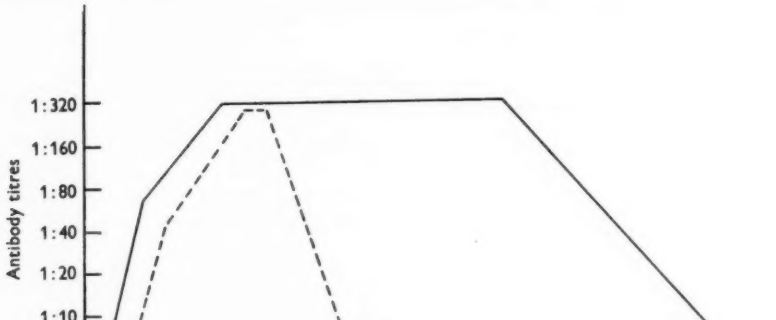

Fig. 1. Diagram showing the formation of neutralizing antibodies in infected and hyperimmunized rabbits. —, infected treated; - - -, hyperimmunized with dead trypanosomes.

Six rabbits between 6 and 10 months old were inoculated intravenously with 1 ml. of *T. brucei* 'O' suspension containing approximately two million organisms killed by 0·5 % formalin. Injections were made every third day and a total of six injections was given. Sera were obtained prior to immunization and during immunization at intervals of 3 days for a period of 1 week, and then once a week until the antibody disappeared. Two-fold dilutions of the rabbit sera were examined for the presence of neutralizing antibody by mixing with a living homologous strain *in vitro* and, after 1 hr. incubation, inoculating into mice. Each dilution of the serum was inoculated into four mice. The results showing the development of neutralizing antibody produced by dead trypanosomes are summarized in Fig. 1. The results of the neutralization reaction with the serum of which the titre reached maximum are shown in Table 2.

Fig. 1 and Table 2 show that rabbits immunized with dead trypanosomes can produce neutralizing antibody, which can reach the same or an even higher titre than one produced in infected rabbits. Maximum titres in rabbits injected with dead

trypanosomes were reached within 35 days and after lasting only 7 days they decreased very rapidly and disappeared after 63 days. In infected rabbits maximum titres were reached within 28 days and started to decrease after 98 days, disappearing completely after 154 days.

Table 2. *Showing neutralization reaction with sera of rabbits injected with six doses of dead trypanosomes* (T. brucei 'O')

	Mortality ratio of mice inoculated with mixture of serum and trypanosomes. Number of rabbits' sera													
Dilution of serum	Pre-inoculation						5 weeks after hyperimmunization						Controls	
	7	8	9	10	11	12	7	8	9	10	11	12	C1	C2
1/10	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	2/2	2/2
1/20	–	–	–	–	–	–	0/4	0/4	0/4	0/4	0/4	0/4	2/2	2/2
1/40	–	–	–	–	–	–	0/4	0/4	0/4	1/4*	0/4	0/4	2/2	2/2
1/80	–	–	–	–	–	–	0/4	0/4	0/4	0/4	0/4	0/4	2/2	2/2
1/160	–	–	–	–	–	–	0/4	0/4	0/4	1/4	2/4	0/4	2/2	2/2
1/320	–	–	–	–	–	–	0/4	0/4	1/4	3/4	4/4	0/4	2/2	2/2
1/640	–	–	–	–	–	–	2/4	3/4	4/4	4/4	4/4	2/4	2/2	2/2

* Accidental death. –, Not tested.

Specificity of neutralizing antibody. After Laveran & Mesnil (1902) demonstrated immune bodies in African Trypanosomiasis, attempts were made to differentiate trypanosomes immunologically. This has been done by the French workers chiefly in goats and sheep, which after recovery from infections resisted reinfection with the homologous species. Unfortunately further tests, particularly in mice, have led to confusion because of the narrow specificity of the immunity.

In order to confirm this view various species of trypanosomes were examined by cross-neutralization reaction. The results are summarized in Table 3.

Table 3. *Showing specificity of neutralizing antibody*

Serum of rabbits infected with	Strains of Trypanosomes				
	T. brucei 'O'	*T. brucei* 1932	*T. rhodesiense* 'B'	*T. gambiense* 'H'	*T. congolense* 'V'
T. brucei 'O'	0	0	0	0	0
T. rhodesiense 'B'	0	0	1/320	0	0
T. gambiense 'H'	0	0	0	1/80	0
T. congolense 'V'	0	0	0	0	1/320
Normal rabbit serum	0	0	0	0	0

Fractions represent the last dilution of serum to show complete neutralization.
0 = No neutralization.

The neutralization reactions of various trypanosomes with homologous and heterologous sera have suggested that neutralizing antibody is species specific, and most probably even strain specific, but further studies with better known strains are necessary to confirm this finding.

The mechanism of neutralizing antibody. Trypanosomes exposed to neutralizing antibody were examined for any morphological changes in comparison with trypanosomes exposed to normal rabbit serum. It was noted that trypanosomes suspended in the serum containing specific antibody showed some reduction in motility in high concentrations of antibody (dilution 1:2). In more diluted antiserum trypanosomes which were neutralized did not show any morphological changes, and were as active as those in the control serum of a normal rabbit. When trypanosomes suspended in antiserum were inoculated intraperitoneally into mice they disappeared in a very short time, presumably being lysed, or phagocytized, while those suspended in serum of a normal rabbit could be detected in the peritoneal cavity for some time.

The fact that trypanosomes are neutralized by homologous antibody is undisputed; but the mechanism by which they do so is not known. Perhaps the action of antibody is similar to that in bacterial and viral diseases.

Thermostability of neutralizing antibody. Three samples of serum of 1 ml. each from rabbits infected with *T. brucei* 'O' and showing neutralizing titre up to 1/160 were diluted 1/2 and heated at temperatures of 56 and 65° C., respectively.

After the sera had cooled, agglutination and neutralization tests were carried out with two-fold serum dilutions. No decrease in agglutinating and neutralizing activity was noted.

Neutralizing antibody in infected guinea-pigs, rats and mice. Experimental infections with *T. brucei* have been produced in monkeys, cats, dogs, rabbits, guinea-pigs, rats, mice and several other animals but those mostly used for maintaining strains are guinea-pigs, rats and mice.

In order to find whether sera of these animals infected with *T. brucei* produce the neutralizing antibody their sera were collected at the peak of the infection and examined for the presence of the neutralizing antibody.

Guinea-pigs. Six guinea-pigs were infected with *T. brucei* 'O' and their sera were collected at weekly intervals and examined for the presence of the neutralizing antibody. The results of the experiment showing the highest titre are summarized in Table 4.

Table 4. *Neutralization reaction between* T. brucei 'O' *and sera of infected guinea-pigs with a homologous strain*

Dilution of serum	Mortality ratio of mice inoculated with mixture of serum and trypanosomes. Number of sera: Pre-infection 1	2	3	4	5	6	21 days after infection 1	2	3	4	5	6
1/2	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4
1/10	–	–	–	–	–	–	0/4	0/4	0/4	0/4	0/4	0/4
1/20	–	–	–	–	–	–	0/4	0/4	1/4	0/4	1/4	0/4
1/40	–	–	–	–	–	–	0/4	0/4	2/4	0/4	3/4	0/4
1/80	–	–	–	–	–	–	2/4	1/4	4/4	3/4	4/4	1/4
1/160	–	–	–	–	–	–	4/4	4/4	4/4	4/4	4/4	4/4

–, Not tested.

Table 4 demonstrates that neutralizing antibody is formed in guinea-pigs. Although the titre is much lower than in rabbits, the strain which is passaged through guinea-pigs undergoes changes similar to those in rabbits. This has been confirmed by isolating a strain after 3 weeks' infection and examining it by neutralization test against the serum collected at the same time as the strain. The strain isolated 3 weeks after infection failed to be neutralized by the antiserum collected at the same time, while the original strain was neutralized completely by this serum in dilution 1/40.

Rats. Sera of six white rats, infected with *T. brucei* 'O' and collected 5 days after infection, failed to show any demonstrable neutralizing antibody.

Mice. On the assumption that no neutralizing antibody is formed during the short-lasting infection in mice, these animals were used throughout the experiment for the maintenance of the strains. However, in order to confirm this assumption an experiment was designed to examine the sera of infected mice 3 days after infection, when animals were usually bled for the transmission of the strains. Sera of infected mice which were treated with 0·2 ml. of 0·05 % of Suramin 1 day after infection were also examined. The results of this experiment are summarized in Table 5.

Table 5. *Neutralization reaction between* T. brucei 'O' *and sera of mice infected with homologous strain*

	Mortality ratio of mice inoculated with mixture of serum and trypanosomes. Number of mice sera											
	Pre-infection						3 days after infection (non-treated)			21 days after infection (treated 48 hr. after infection)		
Dilution of serum	1	2	3	4	5	6	1	2	3	4	5	6
1/1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	0/4	0/4
1/2	–	–	–	–	–	–	4/4	4/4	4/4	0/4	0/4	0/4
1/10	–	–	–	–	–	–	–	–	–	2/4	3/4	2/4
1/20	–	–	–	–	–	–	–	–	–	4/4	4/4	4/4
1/40	–	–	–	–	–	–	–	–	–	4/4	4/4	4/4

–, Not tested.

Table 5 shows that no neutralizing antibody was present in the serum of non-infected and infected mice 3 days after infection, while infected mice which were treated with Suramin 24 hr. after infection and bled 20 days later showed neutralizing antibody only in the serum diluted 1/2. Higher dilutions of the serum failed to inactivate trypanosomes. This experiment has confirmed the observations of various workers that mice, which are killed by trypanosomes in a very short time, are suitable animals for the maintenance of the strain.

Protection test. The term 'protection test' is used when the antibody is injected into a test animal and the protection afforded is measured either by inoculating the animal with the organisms or by exposing it to infection.

Some of the rabbits' sera which were used for the neutralization test were also used for the protection test. In this experiment the protection was measured by inoculating mice with trypanosomes at various periods of time after inoculating the serum. Infections of mice before application of the serum were also investigated.

Prophylactic effect of immune sera. Three groups of twenty mice each were inoculated intraperitoneally with 0·5 ml. of immune rabbit sera and twenty mice were inoculated with the same dose of a normal rabbit serum. Each group of animals was then divided into five smaller groups each containing four mice. The first group of animals was infected intraperitoneally one hour after the inoculation of the serum, the second group was infected 24 hr., the third 7 days, the fourth 10 days and the fifth 14 days after the inoculation of antisera. All animals were infected with the same number of trypanosomes, which were used for the neutralization test. These results are summarized in Table 6.

Table 6. *Showing protection test in mice challenged with* T. brucei 'O' *at various periods of time after the application of the serum*

Dose of serum	Time between application of serum and challenge	Mortality rates: Normal rabbits' serum	Mortality rates: No. of immune sera 4	5	7
0·5 ml.	1 hr.	4/4 (5)*	0/4	0/4	0/4
	24 hr.	4/4 (5)	0/4	0/4	0/4
	6 days	4/4 (6)	0/4	0/4	0/4
	10 days	4/4 (4)	0/4	0/4	0/4
	14 days	4/4 (5)	1/4 (6)	0/4	2/4 (8)

Numbers in brackets show the number of days after inoculation within which the deaths occurred.

It is demonstrated in Table 6 that mice inoculated with the immune serum can be protected completely for a period of 10 days and partially for a period of 14 days.

Therapeutic effect of immune sera. After demonstrating protective antibody in three sera which were able to neutralize trypanosomes in dilution 1/320, tests were made of the therapeutic effect of these sera.

Four groups of twelve mice each were inoculated intraperitoneally with 0·25 ml. of *T. brucei* 'O' suspension showing one to two trypanosomes per microscopical field. Each of these groups of animals was then divided into three smaller groups, each containing four mice. One group of mice was inoculated with normal rabbits' serum, and the three remaining groups were inoculated with immune serum after 1 hr., 6 hr. and 24 hr. (Table 7).

The results summarized in Table 7 indicate that antisera can protect animals against a homologous strain only if they are applied within 1 hr. after infection. When they were applied 6 hr. later they protected some of the animals, but in most cases only the period of incubation was delayed and animals succumbed to the disease within 17 days instead of within 5 days.

Table 7. *Protection test in mice inoculated with* T. brucei 'O' *and tested with antiserum at various periods of time*

Dose of serum	Time between infection and inoculation of antiserum	Mortality rates: Normal rabbits' serum	Mortality rates: No. of immune sera: 4	5	7
0·5 ml.	1 hr.	4/4 (5)*	0/4	0/4	1/4 (10)
	6 hr.	4/4 (5)	3/4 (17)	2/4 (15)	2/4 (17)
	24 hr.	4/4 (5)	4/4 (12)	4/4 (12)	4/4 (15)

Numbers in brackets show the time during which mice died.

Development of antibody resistant (relapse) strains of trypanosomes. Franke (1905) observed that in monkeys infected with trypanosomes causing 'mal de caderas', the parasites were present in the circulation at the time when antibodies could be demonstrated, and he assumed that these trypanosomes were insensitive to antibodies. Similar observations with African trypanosomes were reported by Mesnil & Brimont (1909), and Levaditi & McIntosh (1910).

Because the infecting parasite is able to re-adapt itself or to vary serologically in the body of one host, without any loss of pathogenicity in the variants, the animal body is incapable of ridding itself of an infection with a pathogenic trypanosome although antibodies are produced. The extensive range of antigenic variation of pathogenic trypanosomes propagated in mice, rats and guinea-pigs was studied by Ritz (1914, 1916), Leopold (1928), Russell (1936), Lourie & O'Connor (1937). It appears from the work of these authors that the occurrence of antigenically heterogeneous broods of trypanosomes may appear in a single animal. Unfortunately, studies on antigenic variations carried out by these workers were based either on re-infecting the cured animals or on observations of lysis of trypanosomes by specific sera *in vitro*.

In order to find whether the capacity of trypanosomes to vary serologically is inexhaustible, trypanosomes isolated after the first passage in rabbits were inoculated into the second group of rabbits and then after 4 weeks of infection they were isolated and inoculated into the third group of rabbits. This procedure was repeated several times. Sera from all these rabbits were collected each week and examined by neutralization test and agglutination test against the original strain, which had never been in rabbits, and its variants isolated from each group of rabbits. The results of this experiment are shown in Table 8.

Table 8 indicates that the sera of rabbits infected with strains of *T. brucei* 'O' obtained by passage through rabbits failed to neutralize either the homologous or heterologous variants, but were able to neutralize the parent strain.

It appears from these results that trypanosomes which were passaged through rabbits and constantly exposed to antibodies formed some kind of defensive mechanism or compound which protected them against antibodies.

It was observed that a strain of *T. brucei* 'O', after three to four passages through rabbits, increased its virulence and the course of infection in a rabbit was shortened from 6 weeks to 3 or 4 weeks. When these strains were inoculated into six guinea-

Table 8. *Showing the sensitivity of* T. brucei 'O' *and its variants, obtained by passage through rabbits, to neutralizing antibody agglutinins and to Suramin* (*Bayer* 205)

	Sera of rabbits inoculated with *T. brucei* 'O' and its variants							Agglutinability	Sensitivity to Suramin
	T. brucei	OR 1	OR 2	OR 3	OR 4	OR 5	OR 8		
T. brucei 'O' (parent strain)	1/320*	1/160	1/80	1/160	1/80	1/80	1/160	+	+
OR 1 (*T. brucei* 'O' after first passage)	0	0	0	0	0	0	0	+	+
OR 2 (*T. brucei* 'O' after second passage)	0	0	0	0	0	0	0	+	+
OR 3 (*T. brucei* 'O' after third passage)	0	0	0	0	0	0	0	±	−
OR 4 (*T. brucei* 'O' after fourth passage)	0	0	0	0	0	0	0	−	−
OR 5 (*T. brucei* 'O' after fifth passage)	0	0	0	0	0	0	0	−	−
OR 8 (*T. brucei* 'O' after eighth passage)	0	0	0	0	0	0	0	−	−

* Fractions represent the last dilution of serum producing complete neutralization.
0 = No neutralization. + = Positive reaction. ± = Doubtful reaction. − = No reaction or non-sensitive.

pigs the animals died within 4 weeks, while out of six guinea-pigs infected with the original strain of *T. brucei* 'O' four recovered from infection without any treatment.

It was also observed that when rabbits which were infected with strains passaged through rabbits more than three times were treated with antrypol they failed to respond, even if the doses were doubled or tripled.

Rabbits infected with *T. brucei* 'O' (parent strain) responded in each case to one dose of 2 ml. of 1 % Suramin given intravenously, even when treatment was applied in the last week of infection.

This observation may suggest either the synergetic action of chemotherapeutic drugs and antibodies or that the defensive mechanism of antibody resistant strains is also defensive against some chemotherapeutic drugs. Further studies with antibody and drug resistant strains are necessary to confirm this view.

Absorption of neutralizing antibody and agglutinins by variants of T. brucei. After the experiments on neutralization and agglutination tests with antibody resistant variants, it was decided to test the absorption of neutralizing antibody and agglutinins by antibody resistant variants. Sera of rabbits which were infected with *T. brucei* 'O' and which were able to neutralize *T. brucei* 'O' in a titre of 1/320 were absorbed with suspensions of living variants of *T. brucei* in a concentration containing approximately ten millions of trypanosomes per ml. of fluid.

Suspensions of trypanosomes were added in equal volumes of sera diluted 1:40 and properly mixed. The mixtures were then incubated at 37° C. for 1 hr. and at ±4° C. overnight. Next morning the mixture was centrifuged and the supernatant fluid primarily diluted 1/80 was diluted 1/160 and 1/320 and tested for the presence of neutralizing antibody and agglutinins by the methods previously described. The results are summarized in Table 9.

Table 9. *The presence of neutralizing antibody after absorption with antibody resistant variants and with antibody sensitive original strain of* T. brucei 'O'

Serum no. and titre before absorption	Dilution of serum	Absorbed with	Titre of neutralizing antibody after absorption	Titre of agglutinins after absorption
N38 (obtained from a rabbit infected with *T. brucei* 'O'	1/80	*T. brucei* 'O'	0	1/160*
		OR1	0	1/80
Titre of neutralizing antibody 1/320		OR2	0	1/160
		OR3	0	1/160
Titre of agglutinins 1:1280		OR4	0	1/160
		OR3	0	1/160
		OR5	0	1/160
		OR8	0	1/160

Similar results were obtained with other immune sera obtained from rabbits infected with *T. brucei* 'O' and absorbed with various variants obtained by passage through rabbits.
* Fractions represent the last dilution of serum producing complete neutralization.
0 = No neutralization.

From the results of the experiment (Table 9), it appears that antibody resistant strains obtained after five passages through rabbits, absorbed completely neutralizing antibody and most of the agglutinins.

DISCUSSION

Until now most of the studies on immunity to trypanosomes have been based on the crude method of exposing the individual to reinfection with the homologous parasite. In order to study the nature of immunity to trypanosomes in man and animals it was necessary to determine a more suitable technique for titrating the trypanosome inactivating antibody of immune sera.

The results reported in this paper indicate that the neutralization reaction is the most suitable method, and its use has allowed a closer study of immunity and antigenic changes of trypanosomes than would be practicable by any other current method. It has been demonstrated with a limited number of strains that the neutralizing antibody in trypanosomiasis is species specific and most probably strain specific. Studies carried out on the neutralizing antibody in rabbits infected with trypanosomes showed that the antibody appeared in the blood of rabbits between 5 and 8 days after infection, and reached its peak within 28 days and remained on that level until the untreated animals died. If rabbits were treated during the fourth week of infection the neutralizing antibody persisted in the animals for 22 weeks.

Guinea-pigs infected with trypanosomes produced neutralizing antibody, but the titre of the serum was much lower than in rabbits. Sera of infected mice and rats failed to show demonstrable neutralizing antibody, while sera of infected mice and rats treated with Suramin 48 hr. later show only traces of neutralizing antibody. Sera of rabbits containing neutralizing antibody were able to protect mice from infection against a homologous strain only if they were applied within 1 hr. after infection. However, when mice were inoculated with antiserum, they resisted infection even when they were challenged 10 days later.

The presence of neutralizing and protective antibody in animals immunized with dead trypanosomes indicates that immunity in trypanosomiasis does not necessarily require living trypanosomes. It has been generally accepted that in African trypanosomiasis acquired immunity is always of the nature of 'premunition' requiring the persistence of the living agent in latent form. It seems that the term 'premunition' was used by various workers because they found trypanosomes in the presence of immune bodies, not realizing of course that trypanosomes in the presence of antibodies were antibody resistant (relapse) strains, which were not responsible for the production of antibodies found at the same time as trypanosomes.

It has been shown in the experiments described that trypanosomes passaged through rabbits failed to be neutralized either by the homologous or heterologous sera, which nevertheless were able to neutralize the parent strain.

When an antibody resistant strain was passaged through rabbits the course of infection in rabbits was much shorter than in rabbits infected with the parent strain. It was reduced from 6 weeks to about 4 weeks. It was observed also that when rabbits were infected with antibody resistant strains they did not respond to the treatment with Suramin (Bayer 205), while those rabbits which were infected with the parent strain, or with a strain which was passaged through rabbits once or twice, could be treated even in the later stages of infection.

The increased virulence of an antibody tolerant strain was also noticed by inoculating guinea-pigs, which died within 4 weeks, while those infected with the parent strain in most cases (70%) recovered, though trypanosomes could be isolated in some cases 2 weeks after infection.

Discussing the various points concerning relapse strains it is essential to consider the previous work published on this subject.

Since Franke (1905) first pointed out that pathogenic trypanosomes are liable to undergo a profound immunological change in an infected animal, it has been clearly established by many other workers that this fundamental alteration characteristically distinguishes the organisms of a relapse from those of the initial stages of an infection. Braun & Teichmann (1912) demonstrated that an infected mouse cured by drug treatment is immune for a period against re-infection by the original strain, but is fully susceptible to the parasites derived from a relapse in another mouse which had been infected with the same strain. On the other hand, if a mouse be inoculated with relapse parasites and cured, an immunity follows against the relapse strain but not against the parent trypanosomes. However, in the experiments reported in this paper, it has been demonstrated that early relapse

strains produced neutralizing antibody inactivating the parent strain, but not homologous relapse strains.

Ritz (1914), using Ehrlich's method, demonstrated twenty-two different variants. In a later communication (Ritz, 1916) he concluded that inexhaustible successions of immunologically different variants may appear in the same infection. Leopold (1928), examining relapse strains of trypanosomes by the Rickenberg reaction, concluded that trypanosomes of successive relapses show only a limited variability. Thus, from the first relapses of over fifty mouse infections with *T. brucei*, 96 % of the variants conformed to one or other or both main types.

Raffel (1934), also using the Rickenberg reaction, concluded that the first relapse strains of the same original strain of *T. equiperdum* are the same in different rats. Similar observations were made in this experiment with *T. brucei* in rabbits.

Lourie & O'Connor (1937), by testing the relapse strains of *T. rhodesiense* against the respective sera, obtained from infected mice for their trypanolytic action *in vitro*, demonstrated in the twenty-two relapse strains thirteen distinct variants of trypanosomes. The latter authors were dealing rather with drug resistant variants than with immunological variants, as they obtained their relapse strains by treating a number of mouse infections with subcurative doses of a chemotherapeutic drug 'Halarsol' and tested them against lysins. The method of obtaining lysins in the serum was by infecting ten mice with the particular drug-fast strain, treating with 'Halarsol' 2 days later and bleeding the mice after a further 5 days. There is some evidence that drug resistant strains may be at the same time antibody resistant strains, but more work should be done on this line to confirm this view.

As the infection in mice was of fairly short duration it was doubtful whether sera of mice contained lysins of immune-body nature. However, there can be no doubt from experiments of previous workers already reviewed, that the trypanosomes of relapse differ from those of the initial stages of an infection. It is, however, not entirely clear whether the relapse variants represented genetic mutation or non-genetic modification.

Ritz (1914) gave experimental evidence for the explanation that relapse strains arise in the first place as a mixture of organisms of the relapse and parent type and gradually the former is displacing the latter.

Ehrlich (1911) interpreted the antigenic change in trypanosomes as the response of trypanosomes to the action of antibody. Taliaferro (1941) believed that whatever underlying mechanism is involved in the variations reported for the trypanosomes it is probably the same as when free-living species become adapted to various chemicals.

It appeared from the experiments reported in this paper that trypanosomes exposed to antibody for some time gradually produced some protective mechanism or compound which not only protected them against protective antibodies, but also made the organism inagglutinable and more virulent for rabbits and guinea-pigs. Further studies are necessary on the mechanism occurring in relapse strains of trypanosomes.

It would be interesting to find whether the protective mechanism of relapse strains is chemically related to the mucoid material of capsules present in patho-

genic bacteria. The fact that encapsulated bacteria ingested by leucocytes often resist the digestive enzymes within the phagocytic cell and in due time escape unharmed (Wood & Smith, 1949) may explain why relapse strains escape the defensive mechanism of the host.

SUMMARY

A study of neutralization reaction of trypanosomes with immune sera made against them is recorded, together with a description of the technique.

Neutralizing antibody appears in rabbits infected with *T. brucei* 5 days after infection and reaches its peak (1/320) within 28 days.

The same titre of neutralizing antibody can be produced in rabbits by a series of injections of dead formalized trypanosomes.

Antibody titres persist at high levels for a longer period in animals infected and subsequently treated than in animals immunized with dead trypanosomes.

Neutralizing antibody can be demonstrated in infected guinea-pigs but on a much lower level than in rabbits. Mice and rats infected with *T. brucei* and not treated failed to show demonstrable neutralizing antibody.

Mice infected with *T. brucei* can be protected from infection by inoculation at the same time, or even 1 hr. later, with 0·5 ml. of antiserum showing a high level of neutralizing antibody.

Mice inoculated with the same antiserum showed passive immunity lasting for 10 days.

The evidence that *T. brucei* 'O' after passages through rabbits failed to be neutralized by homologous or heterologous sera which, on the other hand, were able to neutralize the parent strain, suggests that strains after passage through rabbits were not genetic variants, but developed a protective mechanism against immune bodies.

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IMMUNITY IN TRYPANOSOMIASIS

II. AGGLUTINATION REACTION WITH AFRICAN TRYPANOSOMES

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(With 1 Figure in the Text)

Agglutination of trypanosomes was first observed by Laveran & Mesnil (1900) with sera from rats infected with *Trypanosome lewisi*. A few years later the test was carried out with other trypanosomes (Laveran & Mesnil, 1912; Lange, 1911; Mattes, 1912). After these early attempts more studies were carried out on the agglutination reaction in *T. equiperdum* infection (Offermann, 1915; Dahmen & David, 1921) and the reaction has been considered of value in the diagnosis of dourine, but no further work was carried out with the test on African trypanosomiases. Such tests may prove valuable either in the diagnosis or in studying antigenic relationships of various strains.

The present paper describes the results of agglutination of living trypanosomes by the sera from rabbits of which immune status has been established by experimental infection or by hyperimmunization with dead trypanosomes. A few samples of human sera from natural cases of *T. rhodesiense* infection were also examined.

METHOD AND MATERIALS

Strains of trypanosomes

The following strains of trypanosomes were used in this experiment:

Trypanosoma brucei 'O'. (Shinyanga III C.T.R.L., Sukulu, Uganda.)

T. brucei '1932'. (Liverpool School of Tropical Medicine.)

T. rhodesiense 'B'. (Bechuanaland, London School of Hygiene and Tropical Medicine.)

T. rhodesiense 'L'. (Liverpool School of Tropical Medicine.)

T. congolense 'Vom'. (Liverpool School of Tropical Medicine.)

T. gambiense 'H'. (Liverpool School of Tropical Medicine.)

All the above strains were maintained in mice by frequent passage.

Preparation of the antigen

Mice of approximately 20 g. were infected with trypanosomes and bled on the third or fourth day after infection when the parasite counts reach their peak and were between 800,000 and 1,000,000 organisms per ml. of blood. The blood was collected from the cut carotid into modified Alsever's solution (NaCl 1·05 g., sodium citrate 2·00 g., glucose 4·66 g., distilled water 200·00 ml.) made up with horse serum to a concentration of 10%.

The trypanosomes in a mixture of mouse's blood and modified Alsever's solution were left for a period of 1 hr. During that time mouse's red blood cells agglutinated owing to the presence of agglutinins in the horse serum leaving trypanosomes in

the supernatant fluid. This fluid was transferred to another tube and centrifuged. The sedimented trypanosomes were suspended in modified Alsever's solution with horse serum to the required concentration. Trypanosomes were used within 3 hr. after bleeding a mouse, as it was noticed that fresh trypanosomes produced better agglutination than those that had been left for sometime.

Agglutinins. Sera to be examined for the presence of agglutinins were divided into four groups, which comprised:

(1) Sera from rabbits infected experimentally by inoculation with various strains of trypanosomes.

(2) Sera from rabbits hyperimmunized with formalized suspension of trypanosomes.

(3) Sera from human beings naturally infected with *T. rhodesiense*.

(4) Normal human, rabbit and guinea-pig sera used as control sera.

Technique. The test was carried out by mixing 0·2 ml. volumes of serial two-fold dilutions of serum and 0·2 ml. of living trypanosomes suspended in modified Alsever's solution containing horse serum showing opacity equivalent to the No. 10 Brown's tube containing approximately 200,000 trypanosomes per ml. Readings were made after 15 min. standing at room temperature by examining a drop of the mixture from each tube on a slide with a cover-slip under a microscope using $\frac{1}{6}$ in. objective. The test can be simplified by using the direct slide agglutinations reaction, such as is used in bacteriological technique. For more accurate work and for finding the titre of the serum the tube method described above is more reliable. The test-tube agglutination was used throughout this experiment. The following symbols were used to express the strength of reaction:

3+ = More than three large clumps in the microscopical field.
2+ = Two large clumps in at most five microscopical fields.
1+ = One clump after examining several microscopical fields.
± = Small incomplete clumps.
− = No clumping.

Experimental production of agglutinins in experimentally infected rabbits

During the preliminary experiments various antigens and methods including those used by early workers (Lange, 1911; Mattes, 1912) were tested. It was found that dead trypanosomes, whether killed by heat, formalin, or by any other physical or chemical agents, have a tendency to agglomerate, which gives an appearance of agglutination making them, therefore, unsuitable as antigen. The specific reaction was obtained only with living fresh trypanosomes as an antigen.

In order to find how soon agglutinins appear in the serum after infection, six rabbits showing no trace of agglutinins in their sera prior to infection were infected with *T. brucei* 'O'. Three of them were treated with 2 ml. of 1% Suramin (Bayer 205) after 28 days and three were untreated. The sera of these six rabbits were collected at intervals of 3 days and examined for the presence of antibody. The results are shown in the diagram.

The diagram shows the agglutinin titre in rabbits infected experimentally with *T. brucei*. Agglutinins appear after 5 days and the titre then rises gradually, reaching its maximum (1:2580) within 12 days, the titre then remains at the same constant level throughout the period of infection. In an animal treated with Suramin (Bayer 205) [2 ml. 1% intravenously] agglutinins remained for a period of two months after treatment.

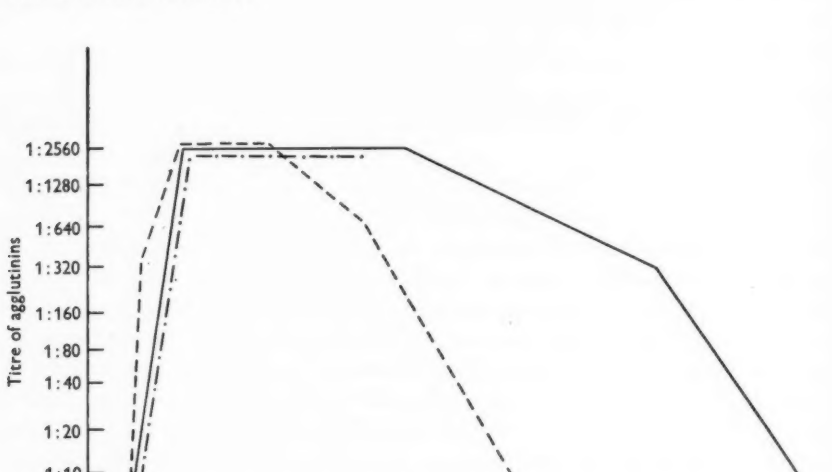

Fig. 1. Diagram showing the formation of agglutinins in infected and hyperimmunized rabbits. ·—·—, infected untreated; —, infected treated; – – –, hyperimmunized with dead trypanosomes.

Production of agglutinins in rabbits hyperimmunized with dead trypanosomes

Similar studies to those on the production of agglutinins by living trypanosomes were carried out on the production of agglutinins by dead trypanosomes. Six healthy rabbits of weight approximately 5–7 lb. were inoculated twice a week for a period of 3 weeks with a heavy suspension of trypanosomes containing approximately 200,000 organisms (No. 10 opacity tube) previously killed by 0·3% formalin. Rabbits were bled once before inoculations and then during hyperimmunization at weekly intervals. The results of this part of the experiment are summarized in Fig. 1.

The diagram shows that agglutinins are produced a week after one inoculation of killed trypanosomes. The maximum of agglutinins is reached after 14 days and remains on that level during the period of hyperimmunization. As soon as the inoculation of dead trypanosomes ceases the titre of agglutinins falls, and within 4 weeks it disappears.

Specificity of the test in identification of trypanosomes

Sera from rabbits collected 3–4 weeks after infection were examined against homologous and heterologous strains of the same species and also against different species of trypanosomes. The results are shown in Table 1.

Table 1. *Specificity of agglutinins in trypanosomiasis*

Serum of a rabbit infected with	Antigen					
	T. brucei 'O'	*T. brucei* '1932'	*T. rhodesiense* 'L'	*T. rhodesiense* 'B'	*T. gambiense* 'H'	*T. congolense* 'Vom'
	Titre of the serum					
T. brucei 'O'	1:640	1:640	1:10	1:10	0	0
T. brucei 'O'	1:640	1:640	0	0	0	0
T. brucei 'O'	1:2560	1:1280	0	0	0	0
T. brucei 'O'	1:1280	1:1280	1:20	0	0	0
T. brucei 'O'	1:640	1:640	0	0	0	0
T. brucei 'O'	1:1280	1:640	1:10	0	0	0
T. rhodesiense 'B'	1:10	0	1:1280	1:2560	0	0
T. rhodesiense 'B'	0	0	1:2560	1:2560	0	0
T. rhodesiense 'B'	0	0	1:640	1:1280	0	0
T. congolense 'Vom'	0	0	0	0	0	1:1280
T. congolense 'Vom'	0	0	0	0	0	1:1280

Controls: Sera from three normal rabbits failed to agglutinate any of the tested trypanosomes.

The results of Table 1 suggest that the agglutination reaction is species specific and not group specific as was reported by Mattes (1912). Although Mattes demonstrated the existence of group reaction he showed that a much higher reaction titre was produced when antigen and anti-serum belonged to homologous groups than when they belonged to heterologous groups. In this work it was demonstrated that sera of rabbits infected with *T. brucei* 'O' agglutinated not only the homologous strain but the heterologous strains and variants of *T. brucei* 'O' at the same dilution of the serum, but failed to agglutinate the heterologous species such as: *T. rhodesiense*, *T. gambiense* and *T. congolense*. Similar results were obtained with sera of rabbits infected with *T. rhodesiense* and with *T. congolense*.

Agglutinins in sera of human patients infected with T. rhodesiense

Sera of four patients collected soon after their admission to the Lumino dispensary, Uganda, and kindly supplied by the East African Central Trypanosomiasis Research Laboratory at Sukulu, Uganda, were tested for the presence of agglutinins. Living *T. rhodesiense* 'B' and *T. brucei* 'O' were used as an antigen. The results are shown in Table 2.

The sera of four patients diagnosed on clinical grounds produced agglutination reaction with titres varying from 1:80 to 1:1280. As the history of infection of the

patients is not known, it is difficult to make any interpretations concerning the titre. The number of sera which were examined were so few that it is also difficult to draw any conclusions, but results appear to be encouraging.

Table 2. *Agglutinins in human patients infected with* T. rhodesiense

Initials, sex and age of the patient	Blood films	Gland juice films	Protein in C.S.F.	Time of collecting serum	Titre of agglutinins: With *T. rhodesiense* 'B'	Titre of agglutinins: With *T. brucei* 'O'
N.O. male 40 years	Positive	—	40 mg. per 100 ml.	1 day after admission	1:80	0
B.M. male 28 years	Negative	Negative	40 mg. per 100 ml.	20 days after admission	1:160	0
B.S. male 30 years	Positive	Positive	40 mg. per 100 ml.	2 days after admission	1:1280	0
A. female 40 years	Positive	Positive	40 mg. per 100 ml.	19 days after admission	1:320	0

Three control normal human sera failed to agglutinate either *T. rhodesiense* 'B' or *T. brucei*.

DISCUSSION

The agglutination reaction described by Lange (1911) and Mattes (1912), who used as an antigen trypanosomes killed by 0·5% formalin, has not found any application in studies of African Trypanosomiasis. This was most probably due to the use of killed trypanosomes, which produced a non-specific reaction. It has been demonstrated that only living trypanosomes maintained by passage in mice in which trypanosomes are not exposed to antibody are suitable as an antigen. Some of the trypanosomes which are exposed to protective antibody for a long time (e.g. by several passages in rabbits or guinea-pigs) become antibody resistant and may lose their agglutinability. This observation has been fully discussed elsewhere (Soltys, 1956).

It appears from this experiment that agglutinins are species specific; agglutinins which were produced by *T. brucei* agglutinated any *T. brucei* strains, provided that a strain is not antibody resistant, but failed to agglutinate closely related organisms such as *T. rhodesiense* or *T. gambiense*. If the preliminary results with a limited number of strains could be confirmed by examination of a large number of strains, the agglutination reaction described in this communication should be of value in identifying the species and particularly in differentiation of *T. brucei* from *T. rhodesiense*. At present only tests on human volunteers are used to distinguish these two species of trypanosomes.

The test described in this paper may also be of value in the diagnosis of African Trypanosomiasis, particularly in latent and low grade infections in which it is not easy to demonstrate trypanosomes by the present clinical and laboratory methods. Further studies in local conditions are necessary to determine the specificity of the test and its value for diagnosis.

SUMMARY

Agglutination reactions between living trypanosomes and sera of experimentally infected rabbits is described.

Agglutinins in infected rabbits and in hyperimmunized rabbits with dead trypanosomes appeared between 5 and 8 days and reached their maximum within 14 days. They persisted in infected untreated rabbits until death, but in infected rabbits treated with Suramin they persisted for only 3 months. In hyperimmunized rabbits the titre of agglutinins gradually fell and disappeared 4 weeks after the last inoculation.

Agglutinins appear to be species specific; sera of rabbits infected with *T. brucei* agglutinated homologous and heterologous strains of the *T. brucei*, but failed to agglutinate heterologous species of trypanosomes. Similar results were obtained with sera of other species of trypanosomes.

Four sera from naturally occurring cases of *T. rhodesiense* infection in man produced agglutination reactions with titre varying from 1:80 to 1:1280 with *T. rhodesiense*, but did not agglutinate *T. brucei*.

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LARVAL TREMATODES (BRACHYLAEMIDAE) FROM THE SLUGS *MILAX SOWERBII* (FÉRUSSAC), *AGRIOLIMAX RETICULATUS* (MÜLLER) AND *ARION LUSITANICUS* MABILLE

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(With 5 Figures in the Text)

In October 1949, one specimen of a brachylaemid sporocyst containing living cercariae was found ramifying over the digestive gland of *Arion lusitanicus*.† (For separation from *A. ater*, see Quick, 1952.) From the same locality, brachylaemid metacercariae were discovered in *Milax sowerbii* (Férussac) and *Agriolimax reticulatus* (Müller). Since 1949 large numbers of slugs from various localities have been examined. No further sporocysts have been discovered but metacercariae have proved to be of relatively common occurrence in some areas. The present paper is concerned with the structure and systematics of the organism, later publications by R. Foster will deal with its seasonal distribution, ecology and its effects on its hosts. It has not yet been possible to rear the adult parasite, attempts to infect chickens, mice, hedgehogs and guinea-pigs having been unsuccessful.

Pavlovskaia (1956) and Dollfus (1954) have commented on the great variability shown in the structure of Brachylaemids. Dollfus, in particular, considers that many adults cannot be specifically differentiated. The organisms described in the present paper belong to the genus *Brachylaemus* (Dujardin, 1843) Blanchard, 1847 and they do not conform to the descriptions given for other species. Because of the present unsatisfactory state of the taxonomy of the family (see Allison, 1943) it has been decided, particularly since the adult stage is not known, not to give the organism a trivial name at this stage.

Source of the material. The parasites were discovered for the first time in October 1949 in slugs collected from an old garden in the North Bailey, Durham City. From that date until 1954, collections of slugs were made at frequent intervals and examined for parasites. Over 3000 slugs were examined of which 95% came from the neighbourhood of the original site. The species were identified from Quick (1947, 1949, 1952). The majority were *Agriolimax reticulatus* but over 300 *Arion ater* (with *A. rufus* and *A. lusitanicus*), over 200 *A. hortensis* and 600 *Milax sowerbii* were included in the sample. Other species examined were, *M. gracilis*, *Arion circumscriptus*, *A. subfuscus* and *Limax maximus*. Apart from eight *Arion ater* or *A. lusitanicus* which contained metacercariae and one specimen of

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† There is much confusion in the literature concerning the status of some species of *Arion*. Frömming (1954) includes *Arion ater* L. and *A. rufus* L., within the species *A.* (*Lochea*) *empiricorum* Férussac but does not mention *A. lusitanicus* M.

A. lusitanicus, which contained the only sporocyst found, infection was confined to the two species *Agriolimax reticulatus* and *Milax sowerbii*. Throughout the period of the investigation the highest infestations were recorded in the garden where the parasite was originally discovered and from which *Arion lusitanicus* was redescribed by Quick (1952).

Morphology of the metacercariae. Four distinct metacercarial stages were discovered and these have been described as the caudal stage, feeding stage, corrugated stage and mature metacercaria. Their distinguishing features are shown in Figs. 1–4. Measurements of the different forms are given in Table 1. The larvae were removed from the hosts and measured alive under cover-glass pressure.

Table 1. *Measurements of metacercariae (mm.)*

		Caudal stage	Feeding stage	Corrugated stage	Mature metacercaria
Body	Length	0·658 (0·320–1·020)	0·917 (0·770–0·990)	1·320 (0·900–1·900)	2·510 (2·040–3·020)
	Width (across ventral sucker)	0·260 (0·200–0·375)	0·535 (0·340–0·900)	0·681 (0·380–0·880)	1·020 (0·860–1·280)
Oral sucker	Length	0·098 (0·060–0·200)	0·183 (0·132–0·220)	0·251 (0·160–0·320)	0·405 (0·310–0·520)
	Width	0·112 (0·080–0·200)	0·180 (0·150–0·200)	0·285 (0·160–0·350)	0·381 (0·240–0·560)
Ventral sucker	Length	0·095 (0·065–0·120)	0·190 (0·130–0·250)	0·251 (0·180–0·350)	0·392 (0·280–0·470)
	Width	0·100 (0·080–0·210)	0·190 (0·100–0·265)	0·290 (0·200–0·370)	0·405 (0·340–0·480)

The single figures represent the means based on samples of twenty individuals, the figures in brackets give the minimum and maximum measurements recorded in the sample. Each vertical column refers to one sample.

(1) The caudal stage (Fig. 1) is the youngest form. Individuals in this condition are probably closely similar to the preceding cercariae, but for the fact that the gut crura are often filled with food material. The principal descending excretory tubules open into an excretory vacuole at the posterior of the body, and are then continued along the length of the caudal appendage as a pair of primitive excretory tubules, each opening by a simple excretory pore at the distal extremity of the appendage.

From many observations on young individuals, decaudation of caudal stage larvae appears to be a gradual process resembling that described for other brachylaemids by Hoffmann (1899) and Ulmer (1951). Towards the end of the caudal stage the primitive excretory canals of the appendage (Fig. 1) become non-functional, and a single median excretory pore opens at the tip of the main body. The caudal appendage is divided into two lateral stems. These two strands of tissue attaching the appendage to the body ultimately break.

(2) The feeding stage (Fig. 2) is characterized by the presence of a densely packed, often refractory, mass within the gut and the caudal appendage has been lost. The excretory vacuole opens by a single pore at the posterior tip of the body.

(3) The corrugated stage (Fig. 3) which follows the feeding stage is so-named because the median parts of the body are extensively furrowed, the furrows extending completely across the animal. The cuticle is only slightly furrowed at the anterior end and the posterior regions often remain smooth. The cuticle may be 15 μ thick in this stage. The gut is usually empty but for a few scattered crystalline masses. The rudiments of the adult gonads are occasionally present as ill-defined darkly staining bodies. Their appearance is usually delayed until the succeeding stage.

(4) The mature metacercaria (Fig. 4) is readily recognizable from its size, smooth cuticle (the corrugation shown in the previous stage having completely disappeared), practically empty gut and, when stained, the prominent gonadial rudiments.

The living infective metacercaria is greyish white, ovoidal, and widest in the region of the ventral sucker. When motionless it becomes concave ventrally and convex dorsally. The cuticle is thick, varying between (approximately) 5 and 12 μ, with a mean of about 9 μ. No cyst or membrane is present.

A circular oral sucker, subterminal and ventral in position, surrounds the mouth, which leads into a short, thin-walled pharynx. The oesophagus is short, bulbous, and thick walled. The gut bifurcates almost immediately, the two branches being arranged as shown in Fig. 4. The ventral sucker is median, and very slightly anterior to the equatorial region.

The reproductive system is obscure and very immature. Genital glands arise in the posterior third of the body, usually appearing as three or four, but occasionally five, darkly staining bodies. All appear similar, even in sections, and there is no obvious differentiation into ovary and testis. The genital pore is circular, lying one-third to one-quarter of the distance between the ventral sucker and the posterior margin of the body. From the region of the gonads arise two indistinct tubes, running parallel to each other to the genital pore; presumably they are the vasa deferentia.

The excretory canal system is shown in Fig. 5. Flame cells are absent. The distal origin of the system is in the lateral body region, slightly posterior to the level of the oral sucker. From here, the primary descending tubule runs posteriorly,

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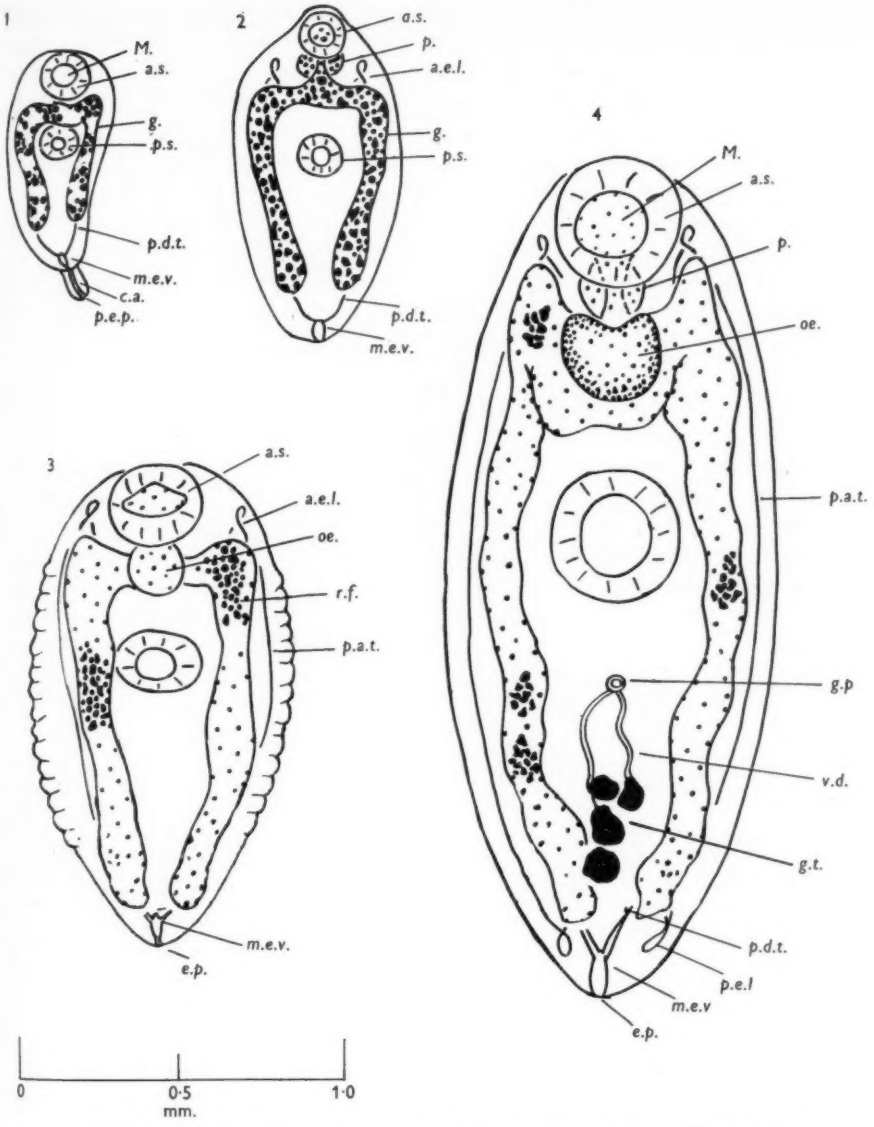
Key to lettering of Figs. 1–5

a.e.l.	anterior excretory loop	*m.e.v.*	median excretory vacuole
a.s.	anterior (oral) sucker	*oe.*	oesophageal bulb
a.t.t.	main anterior transverse tubule	*p.*	pharynx
c.a.	caudal appendage with two primitive excretory canals	*p.a.t.*	primary ascending tubule
		p.d.t.	principal descending tubule
e.p.	excretory pore	*p.e.l.*	posterior excretory loop
e.r.	excretory reservoir	*p.e.p.*	primitive excretory pores
g.	gut	*pr.d.t.*	primary descending tubule
g.p.	genital pore	*p.s.*	posterior (ventral) sucker
g.t.	gonad tissue	*p.t.t.*	main posterior transverse tubule
*l.t.*2.	subsidiary lateral tubule (2)	*r.f.*	residual food mass
*l.t.*3.	subsidiary lateral tubule (3)	*s.a.t.*	secondary ascending tubule
M.	mouth	*v.d.*	vas deferens (?)



Figs. 1–4. The four developmental stages of the metacercaria: 1. Caudal stage. 2. Feeding stage. 3. Corrugated stage. 4. Mature stage.

following the line of the lateral surface of the body, and receiving three subsidiary lateral tubules, two at the level of the ventral sucker and one at a level approximately half-way between the ventral sucker and the posterior limit of the body. At the posterior, the primary descending tubule turns back upon itself with or without a figure-of-eight loop ('the posterior loop of the excretory system' if

present), and runs forward in a convoluted manner to the extreme anterior region. This primary ascending tubule is the only part of the system to be ciliated. The cilia are long and beat rapidly and continuously in a posterior-anterior direction. About the level of the oesophagus the ciliated duct receives another convoluted, but unciliated, tubule, designated the secondary ascending tubule. This arises in the region of the ventral sucker and runs parallel to the ciliated duct until their union.

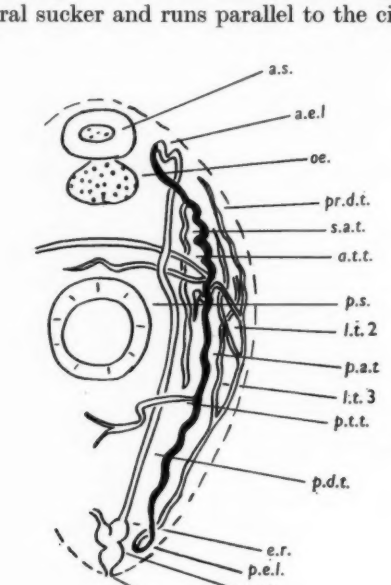


Fig. 5. Excretory canal system of the metacercaria. This is a generalized drawing, right-hand side only, based on the examination of many specimens. The primary ascending tubule which is the only tubule containing ciliated tracts is shown in solid black. *l.t.*1 is not labelled but is shown anterior to *l.t.*2

Slightly anterior to the equatorial region, two further subsidiary ducts join the ciliated duct. The posterior one arises in a median position and runs transversely. The anterior one, designated the main anterior transverse tubule, often appears to traverse the body completely and unite with the ciliated duct of the other side. A further transverse tubule, the main posterior transverse tubule, joins the ciliated duct somewhat posterior to the ventral sucker, having arisen in the mid-body region.

The ciliated duct, on reaching the anterior of the body, loops back upon itself ('the anterior loop of the excretory system') and continues as the principal descending tubule to the posterior extremity of the animal, often with an indentation just anterior to the ventral sucker. This principal descending tubule enlarges in the posterior third of the body and opens into an excretory reservoir. The reservoirs of each side unite to form a single median excretory vacuole, which is

thin-walled and situated between the tips of the intestinal crura. It discharges to the exterior by way of a single terminal pore.

The excretory vacuole is highly contractile. In *Helix* Ringer solution (Pantin, 1946) at room temperature it discharges five or six times per minute.

On removal from the host the parasites exhibit considerable activity in saline solution or tap water, the younger stages being more active than the older ones. The twisting movements observed indicate the absence of any rigid cyst. Continuous movement ceases after about 1 hr., but sporadic movement, especially on mechanical stimulation, is observed up to 5 days after removal from the host.

Location of the parasites. Metacercariae of all stages infest only the renal tissues of the host. The caudal- and feeding-stage larvae are attached to the kidney lamellae by the ventral sucker, the lamellae being distorted and drawn into the cavity of the sucker. The corrugated and mature individuals lie freely in a cavity formed within the kidney as a result of the feeding of the earlier stage larvae. (Details to be published later.)

Longevity of parasites. Within the host, caudal stage individuals live for approximately 4 weeks and the succeeding feeding stages for 6 to 8 weeks. The corrugated stage lasts approximately 1 month but occasionally as long as 2 or 3 months, and the mature metacercaria can survive for at least 4 months including a period of over-wintering. The total life-span of the metacercaria may thus be 8 months, although maturity can be reached after 4 months.

Table 2. *Growth rate of metacercaria*

Developmental stage	Mean size factor (20 individuals) ($mm.^2$)
Caudal	0·127
Feeding	0·406
Corrugated	0·941
Mature	2·524

Rate of growth. In determining the size of individuals, account was taken of changes in shape by multiplying length by breadth (across the ventral sucker) of the animal, the multiplicand being termed the 'size-factor' (Table 2). Alterations in the thickness of the larvae were known from observation to be negligible, and were ignored. It would seem that a uniform growth rate exists throughout metacercarial life.

The sporocyst generation. A single sporocyst found in *Arion lusitanicus* is considered to be the possible forerunner of the metacercaria. Examination of several hundred host specimens for sporocysts have failed to produce further examples of the sporocyst. This sporocyst was a large, branched structure, ramifying over the external surface of the digestive gland of the host. Each branch was slightly enlarged terminally into a thin-walled, sac-like structure containing approximately ten cercariae. The cercariae were structurally indistinguishable from the caudal stage metacercariae described above.

DISCUSSION

Table 3 gives a comparison of the agreed characters of the genus *Brachylaemus* (Dujardin, 1843) Blanchard 1847 with those observed in the present metacercariae. These data indicate that the metacercariae are probably of the genus *Brachylaemus* (Dujardin, 1843) Blanchard, 1847.

If, as seems probable, the described sporocyst was of the same species as the metacercariae, then the likelihood is that cercariae are produced directly from the sporocyst tissues, there being no redial generation. The incidence of sporocyst infection in nature is low, but each sporocyst produces many cercariae and the evidence perhaps indicates that sporocyst and cercarial phases inhabit different hosts from those harbouring the metacercariae.

Table 3

Agreed characters of genus *Brachylaemus*	Observed characters of Metacercaria
1. Length of body 1–7 mm.	1. Length of body approx. 3 mm.
2. Metacercariae possess caudal appendages when young	2. Caudal appendage present for first 4–5 weeks of life
3. Body elongate-oval to elongate-flattened	3. Body elongate-oval
*4. Prepharynx, pharynx and oesophagus present. Oesophagus short	*4. Prepharynx, pharynx and oesophagus present. Oesophagus short
5. Ventral sucker equatorial	5. Ventral sucker equatorial
6. No additional adhesive organ or gonotyle	6. No additional adhesive organ or gonotyle
7. Cuticle smooth or spiny	7. Cuticle smooth; corrugated during one developmental stage
8. Gut caeca reaching almost to anterior limit of body	8. Gut caeca reaching almost to anterior limit of body
9. Genital pore far back	9. Genital pore to one-quarter distance behind ventral sucker
10. Cirrus pouch small or absent	10. Unknown
11. Testes rounded, one behind the other	11. Unknown
12. Ovary between testes, rounded and median	12. Unknown
13. Uterus with ascending and descending limbs. Extends in front of ventral sucker	13. Unknown
14. Eggs small, usually 0·02–0·03 mm. in length	14. Unknown
15. Laurer's canal present	15. Unknown
16. Receptaculum seminalis usually absent	16. Unknown
17. Vitellaria well developed; extend posteriorly only to anterior testes	17. Unknown
18. Excretory vesicle Y-shaped. Tubules extend to anterior limit of body. Ciliated ducts present	18. Excretory vesicle Y-shaped. Tubules extend to anterior limit of body. Ciliated ducts present

* This is the normal terminology used in trematode literature and differs from that employed in the present text.

Of the four developmental stages of the metacercariae, the corrugated stage may be unique (Dollfus, 1954, personal communication). The cuticle of other metacercariae is known to wrinkle with the twistings of the animal, but there is no record of a permanently corrugated stage of some weeks' duration. In the present

species the cuticular corrugations are maintained regardless of the stretchings of the animal's body.

The cuticle is thick, averaging 9 μ but reaching 15 μ during the corrugated stage. It is quite flexible, but very resistant to penetration by intra-vitum dyes. It may well replace the typical rigid cyst which usually surrounds such larvae.

That the main anterior transverse tubule (Fig. 5) appears to connect the two ciliated ducts is of interest. Dujardin (1845), Leidy (1850) and Mehra (1936) have also recorded such cross-connexions in brachylaemid metacercariae. Mehra (1936) claims that in *Harmotrema nicoli* the '. . . two outer trunks of each side are joined by a transverse connection', and that the '. . . two inner trunks are also connected by a transverse vessel at a little distance in front of the ventral sucker'. This latter record is in accordance with the present observations.

The absence of flame-cells may be correlated with the presence of ciliated excretory ducts. The only brachylaemid group in which flame-cells have been definitely observed is the sub-family Leucochloridiinae and this group is diagnostically separated from the rest of the family by the absence of ciliated ducts and the presence of finely-branched tubules.

SUMMARY

1. Metacercariae are recorded from the kidney tissues of slugs. Over 3000 slugs belonging to ten species were examined. Infection was confined to *Milax sowerbii* (Férussac) and *Agriolimax reticulatus* (Müller) apart from a few infected specimens of *Arion ater* (Linnaeus) or *A. lusitanicus* Mabille. Only one sporocyst was discovered and this occurred in *A. lusitanicus*.

2. Four distinct stages in metacercarial development are described—the caudal stage ($0{\cdot}7 \times 0{\cdot}3$ mm.), the feeding stage ($0{\cdot}9 \times 0{\cdot}5$ mm.), the corrugated stage ($1{\cdot}3 \times 0{\cdot}7$ mm.) and the mature metacercaria ($2{\cdot}5 \times 1{\cdot}0$ mm.). The corrugated stage is so-named because of the distinctive appearance of its cuticle.

3. Both the cercaria and metacercaria belong to the genus *Brachylaemus* (Dujardin, 1843) Blanchard 1847 and they probably belong to the same species. Since the life history is not known, no trivial name has been given to the organism.

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THE MERTHIOLATE IODINE FORMALDEHYDE CONCENTRATION TECHNIQUE FOR THE DETECTION OF PARASITIC MATERIAL IN FAECAL SAMPLES

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A report by Blagg, Schloegel, Mansour & Khalaf (1955) on a new concentration technique (the Merthiolate Iodine Formaldehyde Concentration technique or M.I.F.C. technique) for the detection of parasitic material in faecal samples, was published in 1955. It was an advance over other techniques in that it claimed to concentrate trophozoite forms of protozoan parasites. Since we had not used this method in our laboratory, it was decided to carry out a series of examinations using this technique. The primary object was to determine whether a simple technique like the direct saline smear, or a simple concentration technique like Willis's (1921) salt flotation method, or the relatively more complicated M.I.F.C. method was the more efficient diagnostically, particularly with regard to the detection of ova.

MATERIAL AND METHODS

A few faecal samples were taken at random each day from those received by our laboratory for examination for amoebae cysts and ova. All samples therefore were from persons suspected of having parasitic infections. The majority were not fresh specimens, some being received from distant parts of the country. As such, the percentage positive for protozoan parasites is not representative of the true incidence. The consistency of the stools varied from solid to liquid. The majority were of normal consistency. Specimens were prepared by each of the three techniques immediately before examination, i.e. none were preserved and brought to the laboratory. Stains and solutions for M.I.F.C. were prepared according to Sapero & Lawless (1953). The technique followed was that recommended by Blagg *et al.* (1955).

The examination of the slides was carried out independently by persons who were routinely engaged in the examination of stools, and were thoroughly familiar with the appearance of the parasitic material.

STAINING REACTION AND APPEARANCE OF PARASITIC MATERIAL IN M.I.F.C. PREPARATION

As mentioned by Sapero & Lawless (1953) there are two phases in the staining reaction, an early iodine phase and a later eosin phase. No explanation is given by these authors for the combined use of iodine and eosin. Donaldson (1917), who originally suggested the combined use of iodine and eosin for amoebic cysts, merely stated that eosin alone as suggested by Kuenan (1913) is unsatisfactory and not as good as iodine alone. In the M.I.F.C. preparation only the later eosin phase is seen.

Parasitic ova

The general morphological appearances of roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*) are unchanged. The shells stain a dark brown and the contents a salmon pink. These ova are easily identified.

Hookworm ova (*Necator americanus*) remain unstained when viable and are seen clearly as colourless ova in a generally pink background. When viability is lost, the contents take the pink stain, while the shell remains unstained.

The larvae of threadworm ova (*Enterobius vermicularis*) take the pink eosin stain while the shell remains unstained, and with the 2/3 objective they do not stand out as clearly as the colourless eggs of the hookworm.

Tapeworm eggs (*Taenia* spp.) have not been seen in the laboratory since the commencement of this investigation.

Strongyloides larvae stain pink.

Protozoan parasites

Sapero, Lawless & Strome (1951), who originally recommended the M.I.F. method for staining protozoa in stools, state that 'Fixation of the various species of trophozoites occurs without observable damage. . . . Staining of nuclear and cytoplasmic details in trophozoites of freshly passed stools is of good diagnostic quality. The cytoplasm immediately becomes differentiated usually first taking a yellowish coloration which then changes to salmon pink. Nuclear elements stand out in contrast taking a brownish to almost jet black stain. Often granules of the nuclear ring are individually defined.' Our observations agree with the above to a large extent. The fresher the material the clearer the detail. In such material, the finely granular nature of *Entamoeba histolytica*, and, when extended, the clear pseudopodia, are well seen. In *Entamoeba coli* the coarsely granular nature of the cytoplasm and the presence of vacuoles can easily be appreciated. With regard to the nucleus we have observed in very fresh material that the nuclear detail stands out in clear relief, but we cannot confirm that the 'Nuclear elements stand out in contrast taking a brownish to almost jet black stain'. We believe that the brown to jet black appearance is not due to actual staining, but to variation in light refraction at different depths of focusing, because the same ring of nuclear chromatin often appears a deep black, or a pale pink or colourless. The essential staining components of the M.I.F. solutions are iodine from Lugol's solution and eosin in the Tr. merthiolate (as verified from the makers). Donaldson (1917), who originally advocated the combined use of eosin and iodine for staining amoebic cysts, states that 'the cysts have a sharply defined outline and the nuclear structures in the cyst stand out prominently as with the old simple iodine method', and also that reducing the light brings 'the nuclei into relief'. He does not refer to actual staining of the nuclear components. Sapero & Lawless (1953) state that the staining reaction in both direct and vial specimens occurs in two phases: 'an initial iodine stage in which cysts and trophozoites stain from yellowish green to a yellowish brown and a subsequent eosin stage, which gradually replaces the iodine and persists'. The M.I.F.C. preparation has the staining characteristics of the eosin phase owing to the

time taken for preparation. They further state that 'the nuclear membrane appears to stain dark red to jet black while nuclear chromatin remains essentially unstained, as in the simple iodine stain'. The latter part of the statement agrees with our observations, but is contrary to the opinion expressed by Sapero *et al.* (1951) quoted earlier. With reference to the staining of the nuclear membrane, we feel that our early statement applies—that it merely 'appears' to stain, but does not actually stain and that this appearance is due to light refraction. Under the 1/6 objective this same 'black' appearance can be observed in the cell membrane of amoebic trophozoites and the wall of amoebic cysts, but under the 1/12 objective the wall of such cysts can be clearly seen to remain unstained and the cell membrane of the trophozoites to have the same pink colour as the cytoplasm. Careful observation under the 1/12 objective suggests that what essentially occurs is that the cytoplasm and nucleus take a more or less uniform pink stain (since eosin is not an elective stain) and that the various components of the cytoplasm and nucleus are thrown into relief, the extent of relief depending on the different refractive indices. Distinctive staining of the nuclear membrane as such has not been observed. The karyosome and chromatin granules are seen to be colourless or paler than the surrounding cytoplasm and karyoplasm. This is not improbable because they do not have an elective affinity to eosin as they would to a powerful dye like haematoxylin. In fresh material, however, these structures are thrown into relief and can often be seen. In our experience, nevertheless, the karyosome is not always thus visible and in *E. histolytica* it is not always central, but in *E. coli* the karyosome is generally much larger and is so eccentric as to almost touch the nuclear membrane, and the chromatin granules are seen to be larger and irregular in size. The nuclei of very active forms of *E. histolytica*, such as those from cultures, are frequently large and elliptical, so that in observation of the nucleus one would expect to see a great deal of variation. As observed by Sapero & Lawless (1953) 'Nuclear and cytoplasmic elements vary in staining reaction with species, age and stage of protozoan parasites'.

E. coli cysts are generally stained pink and in M.I.F.C. preparations the wall is well defined, the cytoplasm well stained and the nuclei so clear as to make identification simple.

In the stained smear prior to centrifuging the morphological details of *E. histolytica* cysts are very clear. Bars, when present, are pale but clearly seen. In centrifugal M.I.F.C. specimens, however, the cysts' contents tend to retract from the cyst wall and the appearance is not always typically that of the normal saline smear. The latter also applies to cysts of *Giardia lamblia*.

In cysts of *Iodamoeba bütschlii* the glycogen vacuole generally stains a darker pink than the cytoplasm, and later de-stains, so that in the M.I.F.C. preparations these cysts may be mistaken for *Blastocystis hominis*.

Endolimax nana cysts stain pale pink and may be confused with fungal spores.

The detection of all cysts other than *E. coli* may be difficult in M.I.F.C. preparations, unless present in large numbers.

Trophozoites of *Giardia lamblia* stain well when fresh except for the flagella, which remain unstained. Other morphological details, such as the suctorial disk,

nuclei and axostyle are well seen and can be easily identified when fresh trophozoites are present.

Trichomonas hominis from cultures stains reasonably well and the axostyle, nuclei and undulating membrane can be recognized, while flagella remain unstained. We have not seen these organisms in M.I.F.C. preparations from faecal samples and we feel that their identification would not be easy, as they are small and delicate and may easily be overlooked.

It must be mentioned that we used a blue light-filter for carrying out these examinations. Both Donaldson (1917) and Sapero & Lawless (1953) have recommended the use of this filter. They also stress the need for proper preparation of smears, a thin smear being very essential for observing the morphological details of trophozoites.

RESULTS

A total of 543 specimens were examined in this series.

Table 1 shows the numbers of the various species of parasites detected by each technique. Since any one technique did not detect all the positives for each species, Table 2 was compiled to show the numbers positive by combinations of the three techniques.

Table 1. *Ova and protozoan forms in 543 faecal samples as detected by three techniques*

	By M.I.F.C. technique		By Willis's method		By direct smear		By all methods
	No.	%	No.	%	No.	%	No.
Hookworm ova	208	88	165	70	26	11	237
Roundworm ova	161	86	99	53	40	21	187
Whipworm ova	286	91	146	46	29	9	314
Threadworm ova	13	76	11	65	0	0	17
Giardia lamblia cysts	2	8	0	0	22	92	24
Entamoeba coli cysts	36	82	0	0	22	50	44
Entamoeba histolytica cysts	19	59	0	0	19	59	32
Endolimax nana cysts	0	0	0	0	19	100	19
Iodamoeba bütschlii cysts	1	17	0	0	5	83	6
Trichomonas hominis	0	0	0	0	2	100	2
Entamoeba coli	0	0	0	0	1	100	1
Entamoeba histolytica	0	0	0	0	0	0	0

Percentages are in relation to the number detected by all methods.

As seen in Table 1 the M.I.F.C. technique gave the highest diagnostic yield for the detection of hookworm, roundworm and whipworm ova. The incidence of threadworm infection in this country is not known. Cases are met with infrequently. Although the usual method of examination for threadworm ova is by perianal swabbing, it is noteworthy that 13 (76%) of the positive cases were detected by the M.I.F.C. technique.

Eggs of tapeworm species were not detected by any of these methods, nor did we meet with them outside this investigation. As we used two concentration

techniques and as the specimens were all from persons already suspected of having a parasitic infection, this finding tends to show that the prevalence of tapeworm infection is low in this country. It also confirms the observation of Kulasiri (1954) who, though working only on rat cestodes, said that the low incidence of tapeworm infection was not due to faulty laboratory examination, as had been suggested by de Silva (1951).

Strongyloid larvae were not met with in this series.

Our findings are in accord with those of Blagg *et al.* (1955) who state that the M.I.F.C. method recovered a greater number of eggs of each helminth than any combination of other techniques.

Table 2. *Ova and protozoan forms in* 543 *faecal samples as detected by combinations of three techniques*

Detected by	Hook-worm ova	Round-worm ova	Whip-worm ova	Thread-worm ova	*Giardia lamblia* cysts	*E. coli* cysts	*E. histo-lytica* cysts	*E. nana* cysts	*I. büt-schlii* cysts	*Tricho-monas hominis*	*E. coli*	*E. histo-lytica*
M.I.F.C., Willis's and direct smear	11	15	10	0	0	0	0	0	0	0	0	0
M.I.F.C. and Willis's	133	73	112	7	0	0	0	0	0	0	0	0
M.I.F.C. and direct smear	5	10	13	0	2	14	6	0	1	0	0	0
Willis's and direct smear	2	0	2	0	0	0	0	0	0	0	0	0
M.I.F.C.	59	63	151	6	0	22	13	0	0	0	0	0
Willis's	19	11	22	4	0	0	0	0	0	0	0	0
Direct smear	8	15	4	0	22	8	13	19	5	2	1	0
All methods	237	187	314	17	24	44	32	19	6	2	1	0

In contrast to the findings for helminth ova, the M.I.F.C. technique did not give us the same high degree of efficiency in the detection of protozoan parasites. The commonest protozoan parasite seen was *Entamoeba coli* in its cystic form. The largest number was detected by the M.I.F.C. technique. *Entamoeba histolytica* cysts were detected in equal numbers by saline smear and M.I.F.C. *Giardia lamblia* cysts were seen more often by saline smear and reference is made again to the early statement that these cysts are deformed and sometimes difficult to recognize unless present in large numbers. *Endolimax nana* cysts were only seen by direct saline smear, as were trophozoites of *Entamoeba coli* and *Trichomonas hominis*. Trophozoites of *Entamoeba histolytica* and *Giardia lamblia* were not detected by either technique.

The failure to detect trophozoite forms, particularly of amoebae, may be due to several causes. It may partly be due to a certain selection that occurred in the early stages of the investigation when, owing to an oversight, stools which had been shown to be positive for trophozoite forms in the course of routine work in the laboratory were not taken for this investigation. Further, the specimens received by us were several hours old. Blagg *et al.* (1955) state that they examined 'one hundred freshly passed specimens' which were '2–7 hours old' when received in the laboratory. The majority of the specimens received by us were nearer the 7 hr. limit and many over this, so that detection, even in the direct saline smear, would

be difficult owing to death of the amoebae. Faust (1954) recommends that stools be examined preferably within half an hour of evacuation to prevent loss of motility. Motility is the familiar criterion by which amoebae are detected. The M.I.F.C. technique cannot rely on motility, and unless the amoebae are present in large numbers, detection is not likely to be easy, particularly with the 2/3 objective which is used for preliminary examination. Sapero & Lawless (1953), who originally recommended these stains for protozoa, state with regard to the specimens examined by them that 'a majority of faecal specimens examined were heavily populated with both cysts and trophozoites and represented multiple species infection'. The stools examined here are not heavily populated with trophozoites as a rule. Therefore, paucity in numbers may have added to the difficulty of detection. As we did not get any trophozoites of amoebae in our series, we carried out separate tests using heavy cultures of *E. histolytica* and stools, in which cysts or trophozoites were fairly heavy, to make M.I.F.C. preparations. Smears were made before the material was strained through gauze and then from the final M.I.F.C. preparation. In every instance where trophozoites alone were present, the numbers in the former were plentiful, but in the final M.I.F.C. preparation they were very scanty or absent. The probability, therefore, is that the amoebae are held back by the gauze, and this would be made worse by a mucoid stool. Blagg *et al.* (1955) stated that since in forty-six instances protozoan forms were diagnosed by their Technic 1 (which virtually amounts to the examination of the M.I.F. preparation before straining through gauze) and not by their Technic 6 (i.e. the M.I.F.C. technique itself) some of the protozoa may have been removed by straining, but that the higher diagnostic yield obtained by Technic 6 showed that concentration and the greater visibility obtained by removal of particulate matter in the faeces rendered diagnosis of both cysts and trophozoite forms of protozoa easier and more than compensated for the loss. The writer agrees that removal of particulate matter facilitates detection, and that concentration does occur with cystic forms, particularly of *Entamoeba coli* and less so with other forms. The highest figure for concentration—100 %—in Blagg's series was for *Entamoeba coli* cysts while those for *Endolimax nana*, *Iodamoeba bütschlii* and *Entamoeba histolytica* were 94, 94 and 86 %, respectively. Blagg's figures for concentration of trophozoites also showed an appreciable difference between the direct and concentration techniques. However, we have not observed actual concentration of trophozoites in any one of our examinations.

Willis's (1921) salt flotation method was not expected to show any protozoan parasites. As Faust *et al.* (1939) observe, 'Protozoan parasites shrink so much as to be unrecognizable in concentrated salt solution'.

DISCUSSION

The routine method used in this laboratory for the examination of faecal material is the direct smear with saline and iodine. When faecal samples are sent from Ankylostomiasis Campaign inspectors or from school-children, Willis's salt flotation technique is employed. The samples for this series were taken from

specimens that were being reported on by direct smear. Therefore of every 100 positive (by all methods) for hookworm, roundworm and whipworm infection, only 11, 21 and 9%, respectively, would have been actually reported on as being positive, while M.I.F.C. technique detected 88, 86 and 91% of the same infections. The direct smear did not detect any threadworm ova, but M.I.F.C. detected 13 (76%) of the positive cases. The obvious superiority of the M.I.F.C. technique over the direct smear for detection of helminth ova, therefore, needs little comment.

Since Willis's method is of no value in the detection of protozoan parasites, this method cannot be used as a routine in the laboratory, unless used for the detection of helminth ova alone, as is done at present. However, since the M.I.F.C. technique (Table 2) detected more 'positives' than Willis's technique, even for helminth ova, it is proposed to omit Willis's technique and adopt a combination of the M.I.F.C. and direct smear for routine examinations. Table 2 shows the number of cases that would be missed if Willis's technique is omitted. The loss is less than 10% for hookworm, roundworm and whipworm ova, and over this (23%) only for threadworm ova. As perianal swabbing is the recognized method for detection of threadworm ova, and is done on special request, the adoption of the M.I.F.C. technique would increase the present diagnostic yield for threadworm ova as well.

With regard to protozoan parasites a true estimate of the value of the M.I.F.C. technique cannot be gauged from the present results. It is proposed to adopt this technique as a routine method of investigation for all specimens received in the laboratory, together with the direct smear, which it is felt cannot be omitted at this stage. It is also felt that identification of amoebic trophozoites on morphology alone would be a tricky procedure for the average laboratory technician, assuming that actual concentration of trophozoites did occur by the M.I.F.C. method.

It is hoped that future examinations on fresh specimens will give us the same diagnostic efficiency as obtained by Sapero & Lawless (1953) and Blagg *et al.* (1955), although this might not be possible because our specimens are not heavily populated as a rule and the incidence is much lower in this country. Where examination for amoebae is requested, smears are examined without delay. For example, a quick analysis of 500 cases examined by direct saline smear this year showed the following: *E. histolytica* (cysts and trophozoites) 37 (7·4%); *E. coli* (cysts and trophozoites) 24 (4·8%); and *E. nana* cysts 19 (3·86%).

It is certain that heavily populated stools are likely to facilitate diagnosis, while strict attention to the time of centrifuging will lessen the deformity of cysts. The centrifuge used by us was not graduated and took approximately 2½ min. to form the four layers described by Blagg *et al.* (1955).

SUMMARY

The results of the examination of 543 faecal specimens by the Merthiolate Iodine Formaldehyde Concentration (M.I.F.C.) technique, Willis's salt flotation method and by direct saline smear are given.

The appearances of helminth ova and protozoan parasites in M.I.F.C. preparation are described.

The M.I.F.C. method showed a much greater efficiency for the detection of helminth ova over the other two methods. It did not prove as satisfactory for the detection of protozoan parasites. Effective concentration of trophozoite forms was not observed. The possible reasons for this are discussed.

It is proposed to adopt this method in combination with the direct saline smear for routine use in this laboratory.

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STUDIES ON THE BIOLOGICAL CONTROL OF SCHISTOSOME-BEARING SNAILS

PREDATORS AND PARASITES OF FRESH-WATER MOLLUSCA: A REVIEW OF THE LITERATURE

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Although the biological control of medically important snails has been suggested from time to time during the past quarter of a century (Nagano, 1927; Bequaert, 1928; Mozely, 1939, 1951; McMullen, 1952), the subject has, as yet, received but little serious consideration. Upon the initiation of our studies (Chernin, Michelson & Augustine, 1956*a*, *b*) the literature was searched for references to predators, parasites and diseases of molluscs which might furnish leads for experimental investigation.

Specific information on the ecology of freshwater molluscs is all too rare, but the literature pertinent to the predators and parasites of land, marine and freshwater species proved to be both extensive and scattered. Most of the more than 600 references reviewed were obtained from periodicals, manuals and monographs devoted to animals other than snails. Of these, approximately one-third were concerned with organisms affecting freshwater gastropods and pelecypods. Selection of pertinent items has been exercised. Only those references which contained quantitative data pertaining to biological control of medically important molluscs or presenting comprehensive bibliographies are here recorded. Our aim was to ascertain which organisms might possibly offer a means of control for snails rather than to present an encyclopaedic review of the subject.

A few papers dealing in a general way with natural enemies of snails may be mentioned first. Cooke (1895) was perhaps the earliest to review the literature on the parasites and diseases of snails, although his study was concerned primarily with terrestrial and marine species of snails. Similar reviews appeared later by Pelseneer (1926) and Fischer (1951). Pelseneer's paper presents an excellent review of the early malacological literature but, unfortunately, no bibliography accompanies his numerous text citations. Fischer gives complete references in many cases, but some of his examples do not include literature citations and are therefore difficult to verify. Both Pelseneer and Fischer discuss the effects of parasitism on the snail, and dwell somewhat on the pathology which might be caused. Wild & Lawson (1937) prepared an index catalogue of the enemies of land and freshwater molluscs of the British Isles. This material is arranged according to the genera of molluscs and alphabetically according to predators. The information given is helpful, but the paper well illustrates one of the major shortcomings of the literature, namely, a lack of detailed identification of predator and prey. For example, listed as predators of *Anodonta* are bats, otter, water vole, rat, rook,

jackdaw, heron, common eider and the common scoter. Unless one is familiar with the common names of local fauna such information is of small value. The arthropod enemies of molluscs are reviewed by Bequaert (1925) and by Berg (1953). These two papers will be mentioned further under appropriate categories.

The predators and parasites reported from freshwater molluscs are of both plant and animal origin. Those of plant origin include bacteria, algae and fungi. Those of animal origin represent nearly every major phylum. For convenience, they will be reviewed under the following group-headings: (*a*) microbial agents, including algae, fungi, bacteria and protozoa; (*b*) flatworms and nematodes; (*c*) annelids; (*d*) rotifers; (*e*) arthropods; (*f*) molluscs; (*g*) amphibians and reptiles; (*h*) fish; (*i*) birds and mammals. Scientific names are as cited by the original author.

A. MICROBIAL AGENTS

Algae

Since malacologists early focused their attention on the shells of snails, it was natural that organisms appearing to affect this structure were among the first to be noted. Collins (1897) observed that the shells of aquatic snails were frequently encrusted with various species of algae which at times invaded the outer protective periostracum and resulted in erosion and destruction of the calcareous inner portions. This condition is also mentioned by Baker (1916). Goetsch & Scheuring (1926) found specimens of *Anodonta cygnea* and *Unio pictorum* parasitized with *Chlorella vulgaris*. Some of these clams were so heavily parasitized that they appeared green in colour. Specific pathological changes were demonstrated, and experimental infections were established. The authors considered this to be a case of true parasitism and not one of commensalism as observed among various species of *Chlorella* and some other invertebrates.

Fungi

A species of *Catenaria* was observed to invade and destroy the egg masses of *Planorbis guadeloupensis* (Cowper, 1946), and de Meillon (personal communication, 1955) has observed an unidentified fungus which invaded the tissues of unhatched embryonic snails of laboratory-reared *Physopsis*. De Meillon is of the opinion that the fungus probably originated from the hay and grass infusions used in feeding the snails.

Bacteria

Fantham (1923) reported the finding of spirochaetes in the freshwater snail *Isidora tropica* of South Africa. The spirochaetes were not identified nor were they associated with specific lesions.

Berry (1949) reported an epizootic disease with high mortality in the stock colonies of *Australorbis glabratus*, *Physopsis africana* and *Biomphalaria pfeifferi* at the National Institutes of Health, Bethesda, Maryland. The disease was characterized by a swelling of the tips of the tentacles followed by necrosis, haemorrhage and neuromuscular involvements. Normal snails developed the disease when placed in water from aquaria containing diseased snails. Subsequent investigations

indicated that the etiologic agent was a Gram-negative bacterium. So far as we are aware, no additional report on this disease has been published. Experiments conducted in our laboratory indicate that the symptoms described by Berry are not of a specific nature and may be observed in snails exposed to minute concentrations of copper sulphate, urethane, or nicotine sulphate.

Dias (1953*a*, *b*, 1955) reported the isolation of a Gram-variable bacterium, from the ovotestis of *Australorbis glabratus*, which is claimed to have promise as an agent of biological control. It was originally called BET (bacilo de esporo terminal) and later designated *Bacillus pinotti* (Cruz & Dias, 1953). It was described as being a saprophytic bacterium that developed a high degree of virulence after repeated serial passage in *A. glabratus*. Destruction of *A. glabratus* with this bacterium was reported in laboratory and field trials carried out in Venezuela (Texera & Scorza, 1954) and in Egypt (Dias & Dawood, 1955), but these require critical evaluation and confirmation with rigorous controls.

Protozoa

Information on the protozoan parasites of freshwater molluscs, while limited, is more extensive than that regarding bacterial parasites. Freshwater mussels are often parasitized by ciliates of the genus *Conchopthirius* (Ghosh, 1918; Raabe, 1933; Kidder, 1934; Uyemura, 1935) and numerous other species of ciliates have been reported from aquatic gastropods (Leidy, 1877; de Leon, 1919; van den Berghe, 1934; Jarocki, 1935; Kay, 1946). Other than ciliates only a few Protozoa infect freshwater molluscs. Thus, a flagellate, *Trypanoplasma isidorae* was reported from *Isidora tropica* (Fantham, 1923) and *Pfeifferinella ellipsoides*, a coccidian, has been reported from the liver of *Planorbis corneus* (Kudo, 1954). These reports are, however, concerned largely with the morphology of the parasites, and since these Protozoa cause little or no destructive change in the host, they would appear to have no value as agents of snail control. On the other hand, Hollande & Chabelard (1953) report a heavy mortality in laboratory colonies of species of *Lymnaea*, *Planorbis*, *Bythinia* and *Bulinus* due to a flagellate, *Dimoeriopsis destructor*. Hollande & Chabelard suggest the possibility that *D. destructor* might be an effective agent for biological control.

B. FLATWORMS AND NEMATODES

With rare exception all species of trematodes are obliged to pass a portion of their life cycle within a molluscan host. To elaborate fully on the many known species or on the vast literature which deals with them would be beyond the scope of this paper; however, the monographs of Dawes (1946) and of Porter (1938) as well as papers by Sonsino (1892), Frömming (1932), and Cort (1915) serve as an excellent introduction to this subject.

Nagano (1927) attempted to control *Bithynia striatula*, the intermediate host of clonorchiasis in Japan, by exposing the natural snail population to a trematode parasite of ducks. The hope was that the parasite would infect the snails, castrate them, and impede not only their ability to reproduce but also their ability to

become infected with *Clonorchis sinensis*. Brumpt (1941) noted that this experiment was still in progress in 1935 and identified the duck trematode as *Notocotyle attenuatum*. We are not aware whether Nagano actually published the results of his experiment. Recently, Bayer (1954) has advocated the use of non-human trematodes in the control of snail vectors of schistosomiasis in Africa.

The host-parasite relationships between snails and trematodes are extremely variable. Some trematode species are known to cause serious pathological changes in their snail hosts and may even cause death (Sewell, 1922; Porter, 1938; Rankin, 1939; Olivier, Barbosa & Coelho, 1954), whereas other species of trematodes apparently cause little damage to their snail hosts (Rankin, 1939; Pratt & Barton, 1941).

Although Gamble (1910) observed a planarian attacking molluscs, our knowledge of the relationships between turbellaria and molluscs is meagre. With the exception of the papers by Chitwood & Chitwood (1934, 1937) on the snail hosts of nematodes, we have found little material relating to the parasitism of aquatic molluscs by these worms.

C. ANNELIDS

Species of the annelid *Chaetogaster* have been observed in aquatic pulmonates, although it has not been satisfactorily established that these represent cases of parasitism (Baily, 1895; Volz & Frömming, 1936; Krasnodebski, 1936). On the other hand, there is little doubt that several species of leeches act as active and efficient predators of freshwater snails. Species of the genus *Helobdella* (=*Glossiphonia*) seem particularly malacophagous, and many reports attest to their predaciousness (Moore, 1901; Harding, 1910; Elliot, 1917; Miller, 1929; Krull, 1931; Guibé, 1936; Volz & Frömming, 1936; Boisin-Bennike, 1943). Chernin *et al.* (1956*b*) have studied quantitatively the predacious action of the leech *Helobdella fusca* on *Australorbis glabratus*. Their study revealed that under laboratory conditions *Helobdella fusca* was effective, both in destroying the smaller snails and in controlling the growth of snail populations.

D. ROTIFERS

One species of rotifer, *Proales gigantea* Glascott, is known as a parasite of the eggs of pulmonate snails (Glascott, 1893; Stevens, 1912; Nekrassow, 1928). Girard (1908) reported another species, *Proales ovicola*, but this is probably a synonym of *P. gigantea*. The rotifer apparently burrows through the outer egg mass into the egg capsule where it first destroys the snail embryo and then proceeds to lay its own eggs.

E. ARTHROPODS

Three classes of arthropods include species which either prey upon or parasitize freshwater molluscs. These are the insects, arachnids, and crustacea.

Among the insects, certain carnivorous beetles have been recognized as effective predators of pulmonate snails. European malacologists have long been aware that *Dytiscus marginalis* often attacks and destroys the snail *Lymnaea stagnalis* (Williams, 1889; Cook, 1947). Taylor (1894–1900) observed that this beetle is also

capable of biting through the shells of *Planorbis corneus*. The larvae of some Coleoptera may also act as efficient predators of aquatic snails. Okada (1928) noted that the aquatic larvae of *Luciola cruciata* and *L. lateralis* fed upon Japanese species of *Lymnaea*, *Planorbis*, *Melania*, and *Oncomelania*. Impressed with the predatory efficiency of these larvae, Okada suggested that they might be useful in controlling species of *Oncomelania* which serve as the intermediate hosts of *Schistosoma japonicum*. Recently, Hasama (1943) observed that the same species of *Luciola* will feed upon *Thiara libertina*, the intermediate host of *Paragonimus westermani*, in Japan. The favourable reports of Japanese workers resulted in the introduction of Lampyridae into Hawaii to combat the molluscan hosts of *Fasciola* (Fullaway, 1940). So far as the writer is aware, the results of this experiment have not been published. Predatory lampyrid larvae have also been recorded from the Celebes (Blair, 1927).

Certain dipterous larvae may act as predators or parasites of aquatic snails. Entomologists in Europe have noted that species of Sciomyzidae parasitize and often destroy freshwater snails (Lundbeck, 1923; Wesenberg-Lund, 1943). Recently, Berg (1953) has reviewed the literature on this family with respect to its relationship to molluscs. In addition, he has successfully reared species of Sciomyzidae from larvae which fed upon living snails. Berg suggests, on the basis of his experiments and observations, that these insects may be useful in the biological control of undesirable snails.

Other Diptera, on further investigation, may also prove to be effective in the control of snails. Walsh (1863) stated that the larvae of tabanid flies feed voraciously upon aquatic molluscs, and Wesenberg-Lund (1943) has observed tabanid larvae eating species of *Planorbis*. Conflicting accounts regarding the association between midge larvae and aquatic molluscs are to be found in the literature. Barnard (1911) found larvae of *Chironomus* sp. in the mantle cavity of *Lymnaea peregra*, and Mathias & Boulle (1933) reported *L. limosa* parasitized by similar larvae. Additional observations of chironomids parasitizing molluscs have been made by Edwards (1929) and Wesenberg-Lund (1943). Hoffmann (1931), however, examined a mixed series of 5466 snails composed of *Physa ancillaria* Say, *P. heterostropha* Say, and *Lymnaea humilis* Say, but found only five snails which contained chironomid larvae. Jutting (1938) called attention to the larva of the midge *Tendipes* (*Parachironomus*) *varus* Gtgh. which she found in the mantle cavity of *Physa fontinalis*. It is her opinion that the larva is a commensal.

The dipteran-molluscan relationship has been classified by Bequaert (1925) into three ethological types: (1) scavengers, (2) ectoparasites and (3) parasitoids. The examples cited in the above section seem best to fit his third category.

Insects also may serve as a means of transporting smaller species of molluscs from one body of water to another. Kew (1893) cited several records of small bivalves found attached to specimens of *Nepa*, *Notonecta*, and other aquatic Hemiptera, and bivalves have been found attached to the legs or elytra of the beetle *Dytiscus marginalis* (Gardner, 1908; Tomlin, 1908).

Several genera of freshwater mites (*Unionicolidae*) parasitize aquatic mussels. This parasitism, according to Mitchell (1955), may either be transient, involving

only transformation stages, or it may be resident parasitism and include transformation stages as well as nymphs and adults. The literature relating to these organisms is too extensive to cite more than a few of the available references. André & Lamy (1930, 1931) published a general survey of the acarid parasites of molluscs, and recently Viets & Plate (1954) published a comprehensive review of the literature pertaining to the mite parasites of the family *Unionicolidae*; other accounts of parasitism by mites have been noted by Wolcott (1899), Chandler (1934), and Humes & Jamnback (1950).

For the past several years, French workers have experimented with the utilization of Crustacea in the biological control of the snail hosts of schistosomiasis. Deschiens & Lamy (1954, 1955) demonstrated, under laboratory conditions, that crayfish of the genera *Astacus* and *Cambarus* will attack and destroy species of *Physopsis*, *Bulinus* and *Planorbis*. Likewise, an ostrocod, *Cypridopsis hartwigi* will prey upon *Planorbis glabratus* (= *Australorbis glabratus*) and *Bulinus contortus* under laboratory conditions (Deschiens, Lamy & Lamy, 1953; Deschiens, 1954). Lagrange & Fain (1952) have suggested that the malacophagous propensities of the crabs *Potamon lirrangese* and *P. didieri* be utilized in the control of snails. Deschiens, Dechancé & Vermeil (1955) have also experimented with the North African crab, *P. edule*, in the laboratory.

F. MOLLUSCS

One might expect, as in the case of land and marine molluscs, that rapacious snails would constitute a major source of predation upon other freshwater Mollusca. This does not, however, appear to be the case since only a few authenticated records of such predation are to be found in the literature. Most aquatic molluscs are plant or detritus feeders although some act as scavengers and only occasional individuals have been observed to be carnivorous. Acts of predation, when observed, have usually occurred in the artificial confines of aquaria. Under such conditions, Nelson (1865) observed *Physa hypnorum* feeding upon *Lymnaea auricularia* and McLane (1939) reported that *Pomacea paludosa* fed upon and destroyed a small fish, *Villora edwini*. Laboratory and field experiments with the ampullarid *Marisa cornuarietis* (Chernin *et al.* 1956*a*; Oliver-González, Bauman & Benenson, 1956) indicate that this snail will devour both the egg masses and young of *Australorbis glabratus*. This apparently is not due to active predation, but results from the tendency of the young snails to remain on vegetation upon which *Marisa* browses and feeds under laboratory and field conditions. Dawson (1911) reported that individuals of several species of *Lymnaea* are cannibalistic, and Baker (1916) observed that *Lymnaea stagnalis lillianae* occasionally ate *Planorbis campanulatus*.

G. AMPHIBIANS AND REPTILES

Freshwater molluscs supplement the diet of various species of amphibians and reptiles, although it appears that they play but a minor role in the nutritional requirements of these animals. Hamilton (1948) analysed the stomach contents of 434 specimens of the frog *Rana clamitans* Latreille and found that only 3·1%

of the total bulk could be attributed to molluscs. Other North American frogs which feed to a limited extent upon aquatic molluscs are *R. pretiosa* Baird and Girard, *R. catesbiana* Shaw, and *R. pipiens* Shreber (Drake, 1914; Baker, 1916; Stebbins, 1951).

Vercammen-Grandjean (1951) examined the stomach contents of 155 specimens of the African toad, *Xenopus laevis victorianus*, and reported that 60·5 % of them contained molluscs. Since many of the ingested molluscs were of medical importance, the author suggested that these toads be used in control. Lagrange & Fain (1952), however, were unable to confirm satisfactorily Vercammen-Grandjean's observations either in the laboratory or in the field. Among the several species of newts which have been reported to prey on molluscs are *Triturus granulosus granulosus*, *T. g. mazamae*, *Diemictylus viridescens* and *Necturus maculosus* (Ruthven, Thompson & Thompson, 1912; Surface, 1913; Farner, 1947; Evenden, 1948). Lagrange (1951) also observed that a larva of an unnamed pleurodel newt would eat the snail *Planorbis glabratus* under laboratory conditions and suggested the use of this animal for the biological control of snails.

Only a few studies pertain to predation by reptiles upon molluscs. These are based chiefly upon stomach analyses of turtles and on observation of their feeding habits (Surface, 1908; Bishop, 1921; Mell, 1938; Mitchell, 1946), but are not thought to be of significant value in the present context.

H. FISH

Many freshwater fish utilize molluscs as a source of food. Baker (1916) estimated that in the United States molluscs comprise approximately 6 % of the total food fish consumed. The degree to which molluscs serve as a source of food is, of course, variable. Molluscs may constitute as little as 1 % of the diet of the yellow perch, *Perca flavescens*, or as much as 100 % of the diet of the sheepshead, *Aplodinotus grunniens*. Structural modification, particularly of the jaws, make many fish extremely successful predators of molluscs. In addition to Baker's paper, which contains an excellent bibliography, the extensive quantitative studies of S. A. Forbes (1880–1914) should be consulted. Additional reference material is to be found in the publications of the U.S. Bureau of Fisheries.

Biological control of schistosome-bearing molluscs by fish has been suggested by Anderson & Gobert (1924), de Bont & de Bont-Hers (1952), Lagrange (1953), and by Mozely (1951, 1953). The de Bonts's studied the predacious nature of the fish *Serranochromis macrocepha* upon species of *Lymnaea* and *Biomphalaria* and advocate its use in African fish ponds. Their study is of particular interest since fish farming as practised in Africa often provides suitable habitats for the breeding of vector snails (Gillet & Wolfs, 1954). Lagrange (1953) conducted laboratory experiments on several species of African and non-African fish to determine their effectiveness in destroying species of planorbids. Two non-African species, *Umbra pygmaea* (North American) and *Cichlasoma biocellatum* (South American), proved to be more efficient predators than the African species *Tetradon schoutedeni*, *Pelmatochromis kribensis*, and *Tiliapia* sp., although only one species, *Cichlasoma biocellatum*, was capable of destroying snails greater than 3 mm. in diameter.

It is of interest that while *Umbra pygmaea* proved to be a promising control agent under laboratory conditions in Lagrange's (1953) studies, Forbes (1883) found that in field-collected specimens of *U. limi*, a related species, only 5% of the total food consisted of molluscs. Appended to Lagrange's paper is a list of thirty species of fish from the Belgian Congo which have been considered malacophagous. In addition, the East African catfish, *Clarias gariepinus*, has been observed by Mozely (1951, 1953), to feed upon *Physopsis globosa*.

Before a proper evaluation may be made of the possible efficacy of the control which fish may exert on natural populations of snails, quantitative field studies on the food chains of predacious fish must be available. It is also of importance to determine if fish are selective in their choice of molluscan food, or if the availability of a particular species of snail is the determining factor. As the indigenous fish of the endemic areas of schistosomiasis and other trematode diseases have not exerted a noticeable control on the snail hosts thus far, it appears that an introduced species may prove to be most effective. These preceding qualifications are equally applicable to the other groups of molluscan predators.

I. BIRDS AND MAMMALS

Water fowl, particularly ducks (Elgood & Cherry, 1919; Cawston, 1921; Anderson & Gobert, 1924; Humphreys, 1932), have frequently been suggested as a means of controlling populations of undesirable snails, but again there is little experimental or quantitative data to support the feasibility of these suggestions. Hamham (1914) examined the crops of a limited number of wild ducks and reported on the number and species of molluscs found. Mozely (1954) states (without supportive evidence) that: 'In a restricted area, such as a single pond, a flock of ducks is effective in reducing the number of snails, especially the number of dangerous species.' On the other hand, Amberson & Schwarz (1953) believe that a major difficulty in controlling the spread of the snail hosts of African schistosomiasis is their continued dispersal by birds. Dispersal of molluscs by wild ducks and other aquatic fowl has been noted by Kew (1893), McAtee (1914), Coates (1922), and Cawston (1921, 1928).

However, many birds do feed upon molluscs, as is attested by the several thousand records of McAtee (1918), Cottam (1939) and Martin & Uhler (1939). In most instances molluscs serve as a dietary supplement rather than the main food staple. However, Limpkins (*Aramus solopaceus* and *A. pictus pictus*) and the Everglade Kite (*Rostrahamus sociabilis*) feed almost exclusively upon snails of the genus *Pomacea* (Lang, 1924; Nicholson, 1926; Cottam, 1936; Harper, 1936, 1941).

The mammals do not constitute serious predatory threats to freshwater molluscs. Otters, musk-rats and mink have been observed preying upon aquatic mussels (Merriam, 1884; Butler, 1885; Lee, 1886; Anthony, 1928; Hamilton, 1939), but only when other food was not available (Seton, 1909). The water vole, *Arvicola amphibius*, has also been observed feeding upon aquatic bivalves (Tomes, 1850).

COMMENTS

It has become increasingly clear, during the preparation of this review, that there is a great paucity of significant experimental and quantitative data concerning predation and parasitism of freshwater molluscs. Furthermore, most of the reports dealing with the subject have come from casual observations which were seldom accompanied by experiment. Neglect of adequate controls and of the importance of quantitative data has made evaluation of most of the available studies difficult. This matter is further complicated by shortcomings in our knowledge of biological and ecological data concerning the medically important molluscs themselves.

It was the stated purpose of this review to ascertain which organism or groups of organisms might possibly be further investigated as potential control agents for molluscs of medical importance. Unfortunately, present knowledge gives us little rational basis for selecting a specific organism or group of organisms for this purpose. From the little evidence available, it would appear that the groups of mammals, amphibians, reptiles and birds probably offer the least fruitful areas for further study. No general statement can be hazarded with regard to the remaining groups except, perhaps, to focus attention on the bacteria, fungi, and viruses—if for no other reason than that they have received essentially no attention thus far.

In one of the earliest references to the diseases of the Mollusca, Johnson (1850) wrote: 'The diseases of the Mollusca are scarcely known....' It is apparent that during the last century our knowledge, particularly with respect to the freshwater molluscs, has not increased appreciably. Nevertheless, it is hoped that this review may serve those interested in the biology and control of freshwater molluscs as a guide to the literature of the predators and parasites of aquatic gastropods and pelecypods.

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THE INGESTION OF PARTICULATE FAT FROM THE BLOOD BY *TRYPANOSOMA LEWISI* AND *TRYPANOSOMA EQUIPERDUM**

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(With 1 Figure in the Text)

INTRODUCTION

The presence of oil reserves, as seen in many of the free-living Protozoa (Borradaile, Eastham, Potts & Saunders, 1951), suggested that parasitic forms, like trypanosomes, might also contain oil globules (Wotton, 1940). These organisms, which infest the vascular systems of certain mammals, may obtain this lipid from the blood and lymph plasma which they inhabit (von Brand, 1951, 1952). Wotton & Zwemer (1939), Zwemer & Wotton (1944), Wotton & Martin (1951), Wotton & Village (1951), and Wotton & Mosti (1955), in their observations on the direct absorption of fats and oils in mammals, have shown that previously stained lipids may be traced and identified throughout the body. A large part of this fatty material is transported through the circulatory system in visible, droplet form, as well as chylomicra (Gage & Fish, 1924). A study of the ingestion of previously dyed oil by trypanosomes occurring in the blood seemed desirable to elucidate further the physiology of fat absorption.

PROCEDURE

The selection of two suitable host-parasite combinations, as the rat and mouse infected with *Trypanosoma lewisi* and *T. equiperdum* respectively, was decided upon. Heavy blood-stream infestations usually occur with these parasitic Protozoa. Twenty-four young rats, not over 25 days old, were inoculated by an intraperitoneal injection of broth containing a suspension of *T. lewisi*. Eighty young mice were given an intraperitoneal injection of blood containing *T. equiperdum* from a previously infected animal. The average duration of trypanosomiasis in both young rats and mice was 5 days. Some of the animals survived as long as 6–8 days following inoculation with these protozoan parasites. When a sufficiently high trypanosome population had developed in the circulating blood of the rats (Culbertson & Wotton, 1939) and mice, these animals were given orally doses of previously stained cod-liver oil. Usually on the fourth day, microscopical examination of a drop of blood from the cut end of the tail revealed the presence of a large

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‡ New York City. Posthumous.

number of trypanosomes. It was at this time that these animals were fed the Sudan IV coloured cod-liver oil. In this way we were able to provide adequate amounts of dye-identified lipid in the blood.

The red coloured cod-liver oil was prepared by adding Sudan IV, and thoroughly stirring the mixture. Before use it was filtered several times to remove any undissolved dye particles. It was desirable to have a clear solution before adding to various portions of it tryparsamide, atabrine dihydrochloride and penicillin. These compounds were only sparingly soluble in oil, and were carried in the stained cod-liver oil dispersed in a fine suspension. The concentration of these substances was made in such a way that there was about 1·5 grains of drug in 2 ml. of oil mixture.

A test meal consisting of 2 ml. of previously stained cod-liver oil was placed in the stomach of each animal by means of a hypodermic syringe with a large-bore needle which had been blunted. One-half to 1 hr. after feeding, drops of blood, collected from the cut ends of the tails of the experimental animals, were examined microscopically. For each sampling of blood taken, three permanent slides were made for subsequent study. This was accomplished by spreading a drop of blood on a clean slide in the customary manner for making blood films. After drying in air, one slide was mounted directly in Glychrogel (Zwemer, 1933; Wotton & Zwemer, 1935; Wotton, 1937) under a large cover-glass. The second was placed in Romeis's Sudan IV solution to intensify the colour already present in the oil drops. The third was immersed for 20 min. in Baker's Sudan Black B mixture in an incubator at 60° C. Upon removal from the Sudan Black B mixture the slide was rinsed in distilled water and mounted with a large cover-glass. Glychrogel proved to be satisfactory as a non-fat-solvent type of mounting medium with a high index of refraction.

The 104 animals used in the present study were arranged in a series of groups which were observed in sequence. Test meals of previously stained cod-liver oil only were fed to twenty-four young rats and twenty-six young mice. Four days before, these animals had been infected with *T. lewisi* and *T. equiperdum* respectively. In a similar way Sudan IV coloured cod-liver oil containing tryparasamide was fed to eighteen mice infested with *T. equiperdum*. Atabrine dihydrochloride and penicillin also in dyed oil, were given to two groups of six mice each, which had been previously infested with *T. equiperdum*. An additional twelve mice, similarly infested, were given intraperitoneal injections of tryparasimide in aqueous solution. Each injection contained about 1·5 grains of this drug. Culbertson (1939) used germanin (Bayer 205). This was done for a comparison of the trypanosomicidal action of this drug when given orally in oil. A group of twelve mice infected with *T. equiperdum*, as were the others, received no oil or drugs, and served as non-treated control animals.

OBSERVATIONS

Examination with the oil-immersion objective of blood films revealed the presence of previously stained cod-liver oil droplets in the peripheral blood of all animals which had received test meals of this material. About half an hour after feeding,

the first appearance of oil in the blood was detected, and after 1 hr. maximal amounts were seen. The occurrence of previously stained lipid droplets in the blood smears was revealed by the reddish tint imparted to this substance by the dye, Sudan IV. Such coloration was in marked contrast to the pale appearance of the unstained formed elements of the blood and the trypanosomes.

Droplets of coloured oil were seen in close association with many of the simultaneously occurring parasitic Protozoa. In addition, bilobed globules were observed in which one portion outside was connected directly by a small isthmus to another portion of the same oil drop within the trypanosome. The narrow constriction in the oil drops occurred at the site where they passed through the cell membrane of the trypanosomes. This gave to the whole drop of oil an hour-glass appearance (Fig. 1). Red coloured oil droplets of various sizes were observed within the cytosome of many of the trypanosomes. Many of these parasitic Protozoa were seen to contain small accumulations of brownish, granular material. Blood films which were stained in Sudan Black B revealed the same morphological features as those coloured with Sudan IV. A comparison of the cytological picture of oil absorption by trypanosomes showed a marked similarity between *T. lewisi* and *T. equiperdum*.

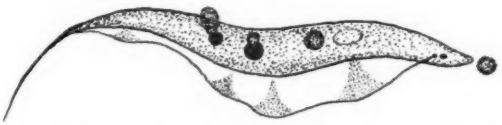

Fig. 1. *Trypanosoma lewisi* from the blood of a young rat. Preparation dried and mounted in glychrogel, unstained. Two drops of previously coloured cod-liver oil may be seen entering the organism. Enlargement about × 5000.

Those mice which received medication with tryparsamide survived the experimentally induced infestations with *T. equiperdum*. It was found that this drug was equally effective in protecting such animals, whether given orally in Sudan IV coloured cod-liver oil, or by the customary intraperitoneal injection of an aqueous solution. Not only did these heavily infested mice fully recover, but their blood was found to be free of trypanosomes several days after the administration of tryparsamide. As was anticipated, atabrine dihydrochloride and penicillin failed to provide any trypanosomicidal action. Infected mice which received these drugs in their oil feedings died at the same time as the non-treated controls. In several instances such animals succumbed in slightly less time.

DISCUSSION

The ingestion of particulate oil globules by trypanosomes in the blood stream may be explained best on the basis of a physical mechanism. Harvey (1937), and Chambers & Kopac (1937*a*, 1937*b*) have accounted for the coalescence of oil drops with *Arabacia* eggs in sea water as a function of reduced surface tension. In the circulating blood, however, there are additional factors which may serve to accelerate or hinder lipid absorption by cellular elements. The action of

agglutinating antibodies may be ruled out as unlikely, because no sticking together of these organisms among themselves was observed. It is more difficult to assess the action of anticoagulating and colloid stabilizing forces which function to prevent gel formation in blood plasma (Davis & Musselman, 1954; Davis & Davis, 1956). Contact in the blood stream between trypanosomes and simultaneously occurring cod-liver oil drops appeared to take place at random. Once, however, a spherule of previously stained oil is adherent to the cell surface an alteration in the plasma membrane may occur at that site. Cameron (1954) has suggested that molecular rearrangements are possible in which lipid molecules present in the cell membrane swing out of position creating localized porosities. The formation of an aperture in the trypanosome surface is supported by the constricted appearance of oil drops observed in transition across this membrane.

If the process of fat and oil absorption be chiefly physical in nature, it might be expected that other substances contained in the cod-liver oil would be carried along intact into the body. Cod-liver oil contains many non-saponifiable fractions like sterols and carotines, as well as neutral fats and free fatty acids. Hewitt (1954, 1956) has shown that triglycerides need not be hydrolysed to be ingested by the intestinal epithelial cells. On the other hand, if extensive hydrolysis and soap formation occurred preparatory to absorption of lipids, the possibility exists of chemical alteration or deletion of non-fatty substances from the oil in the lumen of the alimentary tract. In this connexion a study of the action of tryparsamide proves to be particularly enlightening. Previously it had been necessary to inject this drug parentrally, because it is not effective when given by mouth. The present observations show that this same drug is efficacious in alimentary administration when dispersed in oil. Not only is the modality of administration of tryparsamide helpful in explaining the fate of fatty substances in the body, but also the appearance of the brown granules in the trypanosomes. The accumulation of such granules suggests that the dye is removed from the stained oil and concentrated in small particles. Similar physiological activity has been noted in free-living Protozoa when they separate usable food material from non-assimilable residues and dyes. It is possible that the tryparsamide remains harmless until released in the cytoplasm of the trypanosome; at which time it exerts a toxic effect destroying the organism. Such observations lead us to support the view that lipids are absorbed from the alimentary tract without previous, extensive chemical alteration (Wotton & Zwemer, 1939; Raper, 1949; Frazer, 1952*a*, *b*), and are utilized within the body by differentiated cells (Wotton & Village, 1941; Wotton & Mosti, 1955), as well as by two species of parasitic trypanosomes that had been introduced artificially.

SUMMARY

The ability of certain species of trypanosomes (*T. lewisi* and *T. equiperdum*) to ingest previously stained oil drops which occurred simultaneously in the blood has been observed.

Tryparsamide incorporated in dyed (Sudan IV) cod-liver oil,when administered orally, was found to be effective against *T. equiperdum* infections in mice.

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ON A NEW PARAMPHISTOMID TREMATODE, *GIGANTOCOTYLE LEROUXI* SP.N. FROM THE STOMACH OF THE RED LECHWE, *ONOTRAGUS LECHE* FROM NORTHERN RHODESIA

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(With 2 Figures in the Text)

A score of these large amphistomes were found in the collection made by Dr P. L. LeRoux, to whom we wish to express our gratitude for the privilege of examining them, and to Professor J. J. C. Buckley for his interest in the work. The characteristically large posterior sucker and its muscular structure places this amphistome in Nasmark's *Gigantocotyle*.

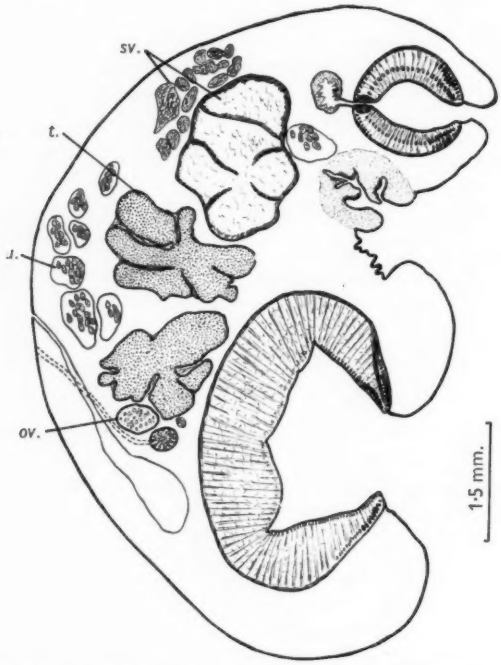

Fig. 1. Sagittal section of *Gigantocotyle lerouxi* sp.n. *ov.*, ovary; *sv.*, seminal vesicle; *t.*, testis; *u.*, uterus.

The worm is about 11–12 mm. long and 3·6–4·4 mm. thick. The dorsal line is semicircular, causing both the oral and posterior suckers to open ventrally. The tissue around the posterior sucker in some is smooth, while in others it is very

wrinkled. The rounded posterior sucker is 3·9–4·2 mm. in diameter. It gives a ratio in relation to body length 1:2·6–3·0. Its muscular arrangement agrees with Nasmark's *Symmeri* type. A count of the circular layer of muscle varies. The d.e. 1 varies from 12 to 17; the d.e. 2 from 5 to 7; the d.i. from 30 to 31; the v.i. from 36 to 50 and the v.e. 15. Occasionally, in some perfectly sagittal sections, the dorsal interior circular layer is almost continuous round the sucker to meet the ventral group, and it is only with some discretion that one says the lot stops at a certain

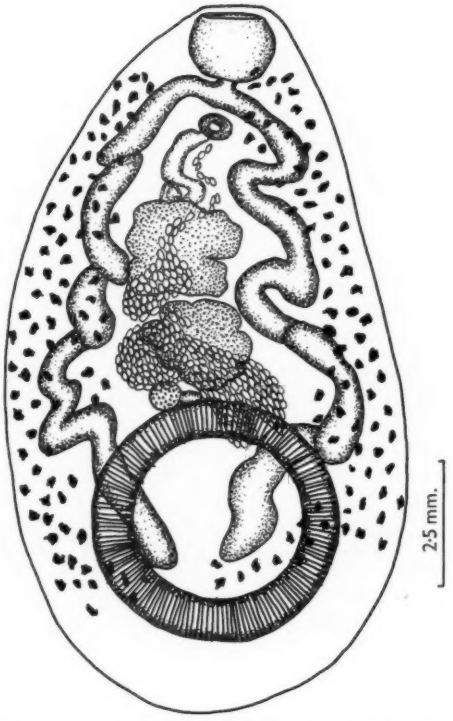

Fig. 2. Whole mount of *Gigantocotyle lerouxi* sp.n.

point. This is made the more difficult when the bundles decrease in size gradually. The pharynx which is of *Explanatum* type is 1·3–1·5 mm. long. The exterior circular layer is well developed and meets the anterior sphincter. The oesophagus is 0·25–0·44 mm. long, and is devoid of a sphincter or bulb. The long undulating caeca run dorso-ventrally, making three turns each dorsally and ventrally, to end close to the posterior tip of the body.

The deeply lobed, diagonally placed testes meet medially. They are about 2–2·5 mm. long and 2·5–3·0 mm. dorso-ventrally. The genital atrium is of *Explanatum* type. The ovary lies immediately behind the posterior testes and is about 1·1 mm. in diameter, with the large Mehlis gland posterior to it. The uterus follows a course dorsal to the testes. The eggs are $80 \times 130 \mu$.

The excretory vesicle is behind the posterior sucker and the excretory canal is directed upwards and forward to open on the medial dorsal side. The Laurer's canal is directed anteriorly, crosses the excretory canal and opens slightly to the side, a little posterior to the excretory pore.

We propose to name this new species, *Gigantocotyle lerouxi* in honour of the collector Dr P. L. LeRoux.

Types: in the Helminthological Collection, London School of Hygiene and Tropical Medicine. *Host: Onotragus leche*, mammal. *Locality:* Northern Rhodesia. *Habitat:* abomasum.

DISCUSSION

According to Nasmark's system of classification, this new stomach amphistome resembles *Gigantocotyle formosanum* in that they both have an *Explanatum* type pharynx, *Symmeri* type posterior sucker and *Explanatum* type genital atrium. This new parasite, however, differs from *Gigantocotyle formosanum* in its larger size and geographical distribution. The most important differential character is seen in the long sinuous caeca which traverse the lateral margins in dorso-ventral undulations and reach the posterior end of the worm, whereas in *G. formosanum* the caeca are short and straight and never extend much below the anterior border of the posterior sucker. This character makes it easy to differentiate the two species.

Our collection of *G. formosanum* from the abomasum of the domestic buffalo in Canton, China, is the first record of this parasite on the Chinese mainland.

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P.S. Since this paper went to the press, the author has received from the collector more of these flukes. One of the jars had several hundred, all coming from the type host and locality.

A MICROSCOPIC STUDY OF *PASTEURELLA TULARENSIS* IN THE HUMAN BODY LOUSE*

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(With Plate XVIII and 1 Figure in the Text)

INTRODUCTION

This paper presents the results of the third phase of an investigation into the relationship between the tularemia organism, *Pasteurella tularensis*, and the human body louse, *Pediculus humanus humanus* L. These studies dealt with a thorough histological study of lice in various stages of infection to determine the fate of *P. tularensis* in the louse.

In a qualitative study on the ability of the tularemia organisms to survive in lice and louse faeces, it was shown that both substrates harbour the organisms for varying periods ranging up to 53 days (Price, 1954). It was subsequently demonstrated that the human body louse is susceptible to infection with *P. tularensis*, the percentage retaining infection over a period of time depending upon the number of organisms accessible to the louse in its blood meal (Price, 1956). By using quantitative methods, it was noted that an increasing percentage of the lice lost its infection with passage of time, whereas in some of the lice the bacteria multiplied extensively.

A histological examination of this nature is important, both to supplement the picture of louse infection obtained in the previous works and to afford a means of comparison with lice infected with the various rickettsiae pathogenic to man. Fundamental to the definition of rickettsiae is the intimate association with arthropod tissues, and *P. tularensis* is the only bacterial pathogen of humans which shows a predilection for intracellular development within the arthropod host. Since Weyer (1954) has made a series of comprehensive histological studies of *Pediculus humanus humanus* infected with the rickettsiae pathogenic to humans, it is of interest to determine in what respects an infection of body lice with *Pasteurella tularensis* resembles these findings.

Only one other study of tularemia in the tissues of arthropods is known to the writer. Francis (1927) found ample cellular infection in the epithelium of the digestive tract and occasionally in the Malpighian tubules of *Dermacentor andersoni* Stiles and *Cimex lectularius* L.

MATERIALS AND METHODS

The strains of body lice, *Pasteurella tularensis*, and white mice used throughout the study were the same as used previously by this worker (Price, 1954). The agar used for the cultivation of this organism was glucose-cysteine blood agar (GCBA)

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prepared essentially after the method of Downs, Coriell, Chapman & Klauber (1947); in order to reduce the growth of bacterial contaminants, 6250 units of penicillin and 25 mg. of sodium sulphadiazine were added to 500 ml. of medium.

Lice were infected by the method of Snyder & Wheeler (1945), utilizing the marginal ear vein of a rabbit for an intravenous inoculation of a massive dose of the infectious organisms. All lice used were starved for 12–24 hr. before infection. They were applied to the rabbit immediately after the injection of the infectious material and allowed to feed for 20–30 min. After this time the lice were removed and all lice which obviously had not fed were discarded. The infected lice were stored in desiccating jars at 29° C. and a relative humidity of 60 %, using the sulphuric acid method of Solomon (1951) for humidity control.

A standard procedure was adopted for the quantitative determination of the number of organisms in each louse. The individual louse was placed in a sterile mortar with 1·5 ml. sterile saline and thoroughly macerated. This original suspension was considered as a 10° suspension, and 1·0 ml. of it was transferred to a 99 ml. saline blank, with 0·2 ml. being used to make duplicate 10° plates, 0·1 ml. per plate. Duplicate plates similarly were made at 10^{-2} and a further 100-fold dilution enabled duplicate plates to be made at the 10^{-4} dilution. When pooled lice were plated, a similar procedure was adopted. For all plate counts, the GCBA plates were incubated at 37° C. for a minimum of 3 days before being counted. The accuracy of this plate-count method was tested statistically and found to be adequate (Price, 1956).

The infected lice were fed once daily on normal rabbits, this interval being kept as near 24 hr. as was possible. No rabbit was used for more than the 48 hr. period following the first feeding of infected lice. In this way one rabbit would provide at most three consecutive meals. To test for a possible early circulation of *P. tularensis* each rabbit was bled from the marginal ear vein immediately before the second and third feedings of the lice. Two white mice were inoculated with 0·5 ml. each of this blood. The mice were observed daily until the fifteenth day at which time, if still living, they were sacrificed and autopsied. The lymph nodes, spleen, and liver of every mouse were examined grossly for pathological changes indicative of tularemia and plate smears of the spleen and heart blood were made. The rabbits were similarly observed and autopsied after infection-induced or sacrifice-induced death. Gram-stain preparations were made of any apparently positive plates from these animals.

All lice used for the preparation of serial sections were fixed in Gilson's fixative after they were killed by a 5 sec. immersion in boiling water. The heads and legs were removed after killing and before fixation. The method of dehydration and embedding was based mainly on the *n*-butyl alcohol technique of Stiles (1934). At the suggestion of Dillon (1954), phenol was added to the higher alcohol solutions to give a concentration of 4 %.

The lice were cross-sectioned at 4 or 5μ thickness. The usual method of affixing the tissues to the slides was followed with the exception of the substitution of a rabbit blood serum adhesive (Priman, 1954) for the egg albumin formula.

Sections were stained in Giemsa stain following Lee (1950), whose procedure was

altered by reducing to 20 drops the amount of Giemsa stock and increasing to 4·0 ml. the volume of pure methyl alcohol for each 50 ml. distilled water. The slides were left in the stain for only 4–6 hr., followed by a 20–25 min. differentiation in 95% ethyl alcohol.

MICROSCOPIC STUDY OF INFECTED LICE

Approximately 5000 lice of mixed ages were infected to obtain material for the preparation of serial sections. To ascertain the course of the infection within these lice, counts of pools of five lice were performed. Ten pools tested on the initial day were 100% infected with a maximum of 16×10^6 and a minimum of 6×10^6 organisms per pool, the average being 10×10^6. Ten pools tested on the seventh day after infection were still 100% infected, the maximum being 80×10^6, the minimum 5×10^6, and the average 27×10^6 organisms per pool. Only five pools were tested on the fourteenth day, 80% of these being infected with a maximum of 20×10^6 organisms per pool. These results indicated some bacterial multiplication within the lice of this infected colony.

On the sixth and thirteenth days, 27 and 25 female lice, respectively, were placed individually into faecal collectors after their meal for that day. The 24 hr. accumulation of faeces for each louse was ground individually in 0·6 ml. sterile saline and the undiluted suspension plated in duplicate on GCBA plates, 0·1 ml. per plate. Faeces and lice were numbered correspondingly, and the latter were fixed and dehydrated individually. The lice were then grouped into three groups depending on whether the plates made from their faeces exhibited: (1) a growth too heavy to count easily (23 lice); (2) 1–300 colonies per plate (21 lice); (3) no *P. tularensis* colonies (eight lice). Ten lice, randomly selected from each of the first two groups, and the eight lice of group 3 were sectioned.

All of the lice examined from the group with heavily infected faeces showed excellent development of tularemia-like organisms in the epithelium of the midgut. Of the total of 3203 sections inspected, 376, or 11·7%, showed intracellular infection with these organisms. Of the 10 lice from the second group, seven showed infected gut cells, and of these seven, only two had as extensive infection as the preceding group. Only 116, or 3·9%, of the 2981 sections of these lice revealed infected cells. Significantly enough, all 2134 sections of the eight lice examined from the uninfected-faeces group showed no cells with tularemia-like organisms. Since the above sections were cross-sections, it should be emphasized that in the heavily infected lice there was more infection along the lateral axis as well as the longitudinal axis.

Lice for the main histological study were selected from the colony of infected lice maintained for a period of 35 days (Price, 1956). Their manner of selection was as follows: 19 lice on the first day, 21 on the fourth day, 25 on the tenth day, 27 on the seventeenth day, and 30 on the thirty-fifth day. Controls were obtained by selecting and similarly processing the following numbers of lice from the normal stock colony: 10 first-instar lice on the first day, 13 second-instar lice on the fourth day, and 30 adults (14 males, 16 females) several days after the thirty-fifth day.

Serial sections of 19 first-instar lice prepared 1 day after infection were examined.

Of these lice, 17 showed *P. tularensis* exclusively within the lumen of the midgut (Pl. XVIII, fig. 1). These organisms were found to range all the way from the caeca to the portion of the digestive tract approaching the rectal papillae. Often they were noted to be more concentrated toward the most posterior portion of the midgut. Where the gut content clouded the observation of the major portion of the lumen, organisms usually could be seen near the periphery of the lumen adjacent to the epithelial cells.

Of the 21 lice examined from the group fixed 4 days after infection, 16 were infected with *P. tularensis*. All 16 of these revealed organisms present in the lumen of the midgut. Four of these 16 lice also showed some degree of intracellular infection, this infection being very light and limited to one or several cells in the region of the juncture of the oesophagus and the midgut; these cells were dorsal in position and occurred in the groups of cells adjacent to the entering oesophagus (Pl. XVIII, fig. 2). The lack of distension of the infected cells and the paucity of the *P. tularensis* gave a clear picture of the early stages of infection.

Of the 25 lice fixed 10 days after infection, 14 were tularemia infected. Four of these showed organisms restricted to the gut lumen. The 10 remaining lice which demonstrated intracellular infection could be placed in three arbitrary groupings according to the degree of infection which they showed. Four revealed only a very limited infection of one to a few cells, dorsal in position and adjacent to the previously mentioned oesophageal juncture with the midgut. They presented a picture quite similar to that of the few lice found infected intracellularly after 4 days.

Four other lice showed a more extensive infection which, however, did not extend beyond the bounds of the gut epithelium. The infection at the oesophageal juncture involved more cells and showed greater distension of those cells involved. Besides this infected region, other areas of the midgut became involved occasionally. Infected single cells or groups of cells extended forward into the caeca (Pl. XVIII, fig. 3) and as far back as half way through the midgut.

The two remaining lice demonstrated not only an extensive infection of the midgut epithelium but also an invasion of their body cavity by *P. tularensis*. Observation of the widespread infection and the accompanying distortion and obliteration of the infected cells made the source of entrance into the haemocoele fairly clear. In one louse, with a very heavy infection spreading anteriorly and posteriorly from the oesophageal juncture, an area of the caecal wall was noted where the organisms apparently had broken through into the haemocoele. Although no such specific point of entry was established for the other louse, the heavy infection and cell destruction in the oesophageal-juncture region probably provided the site of entry to the haemocoele. The latter louse was unusual in showing an extensive infection of the midgut epithelium toward the posterior portion of the gut. This was the only instance of such an infection among all lice examined in the experiment.

The *P. tularensis* free in the haemocoele appeared to be dispersed evenly throughout the haemolymph. The bacteria were found lying between muscles, fat bodies, reproductive organs, and other internal structures. An occasional blood cell was encountered that appeared to contain a number of the organisms in its cytoplasm.

Such presumably phagocytic cells were more conspicuous toward the posterior of the abdomen.

Of the 27 lice taken from the colony 17 days after infection, only 10 (five males, five females) showed the presence of *P. tularensis*. No differences in infection due to sex were apparent. Seven lice had tularemia organisms visible in the gut lumen and two had organisms present in the haemocoele. As with the 10-day lice, several (3) had a very light infection near the oesophageal-midgut junction, others (5) showed a much more extensive infection of the midgut epithelium, while in the remainder (2) the haemocoele as well as the gut cells were involved. The infection of all of these predominated in the region of the dorsal cells around or near the junction of the oesophagus to the midgut. In the more heavily infected lice the infection spread both forward into the caeca and posteriorly farther into the midgut, but no infected cells were seen in any lice beyond the anterior third of the midgut.

An examination of the serial sections of 30 lice (15 males, 15 females) killed 35 days after infection revealed only three infected lice (two males, one female). All three showed numerous organisms in the gut lumen, and although only one displayed the presence of *P. tularensis* in the body cavity, they all exhibited such heavy infection of the midgut epithelial cells that haemocoele invasion was presumably imminent (Pl. XVIII, fig. 4).

Table 1 summarizes the data presented above. A comparison of the percentages of lice in the various stages of infection shows that, with the passage of time after infection, there is a tendency for the *P. tularensis* to shift from the lumen of the gut to the gut epithelium and eventually to the body cavity.

Table 1. *Progress of tularemia infection in lice*

Days after infection	Total lice examined	Percentage of lice infected	Percentage of infected lice with: Lumen infection only	Light midgut infection	Heavy midgut infection	Heavy midgut and haemocoele infection
1	19	89	100	0	0	0
4	21	76	75	25	0	0
10	25	56	29	29	29	13
17	27	37	0	30	50	20
35	30	10	0	0	67	33

The infection of the epithelial cells of the midgut occurred predominantly in its anterior portion. To determine how prevalent this tendency was, a tabulation of the percentages of lice showing cellular infection of the various regions of the gut was made. The numbers used in the designation of the gut regions infected were a series of landmarks proceeding posteriorly from the anterior margin of the thorax (0). Subsequent numbers refer to the beginning of the gastric caeca (1), the juncture of the oesophagus and the midgut (2), the first (3), second (4), third (5), and fourth (6) abdominal spiracles, the anterior portion of the recurved midgut (7), the most posterior portion of the midgut (8), and the end of the abdomen (9). A total of 44 lice with intracellular infection of the midgut obtained from several

experiments was employed. The percentages of lice infected in the particular gut regions are as follows:

Regions of gut								
0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
0	66	98	36	16	2	2	5	0

It is evident from this that infection of the anterior third of the midgut predominated among the infected lice and only rarely was infection noted in the posterior two-thirds.

As would be expected, cells in all stages of infection were found. Cells in the early stages of infection showed very little if any distension and appeared normal in shape. However, advanced infection gave a typical picture of cellular swelling or ballooning (Pl. XVIII, fig. 3). The tularemia organisms uniformly filled the entire cytoplasm of the cell, causing the cell to become distended and to protrude into the lumen of the gut. The consistent occurrence of intact nuclei made it appear that the *P. tularensis* did not invade these but multiplied only in the cytoplasm of the infected epithelial cells. Occasionally an infected epithelial cell was noted free in the gut lumen. Rarely cells were observed, which seemed to have burst, with their walls bordering on the lumen apparently having ruptured and released their contents.

An examination of the control slides showed no organisms, either intra- or extracellular, that could be confused with the *P. tularensis* observed in the infected lice.

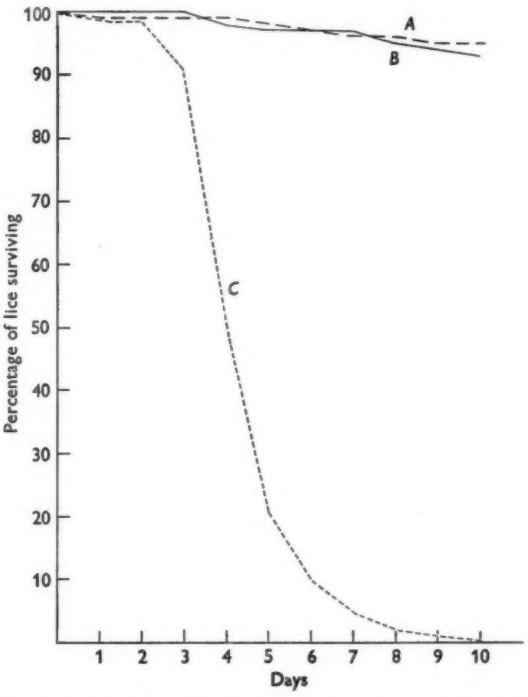
INOCULATION OF THE LOUSE HAEMOCOELE

With the observation in several instances of *P. tularensis* in the haemocoele of the louse, it became of interest to determine what effect, if any, the presence of the organisms in this cavity had on the longevity of the louse. The inoculation of tularemia organisms directly into the haemocoele offered an approach to this problem. The lice used for the body cavity inoculation of *P. tularensis* all were within their first 2–4 days of adulthood. They were inoculated with a fine pin inserted near the lateral margin of the ventral integument at the level of the fifth abdominal segment. This pin was dipped into a 24 hr. GCBA culture of the tularemia organisms immediately before each abdominal puncture. A simple and rapid insertion and withdrawal of the pin was made in the hope of avoiding as much damage to internal structures as possible. After inoculation, the lice were stored at 28° C. and 60% relative humidity, and were fed once daily on normal rabbits. In this manner 150 lice were inoculated with *P. tularensis*.

Two control groups of lice were maintained along with this infected group. One control consisted of 108 lice which had been punctured with a sterile pin in exactly the same manner as the above infected group. The second control group contained 152 lice which had undergone no special treatment. Both control groups were maintained under the same conditions as the infected lice. At each feeding a count of both dead and surviving lice was made; in this manner every louse was accounted for. The criterion for dead lice was established to include not only those lice which

were actually dead but also those which were so near death that only a very weak peristalsis or an occasional leg tremor was present.

For the first 2 days after the inoculation of the lice, all three groups showed similar survival. However, the death of 11 lice in the infected group on the third day heralded the onset of a rapidly progressing mortality within that group. Sixty-one deaths occurred on the fourth day, 45 on the fifth day, and excessive mortality continued until all lice were dead on the tenth day. Both control groups showed similar and almost perfect survivorship. Text-fig. 1 illustrates the findings of this experiment.



Text-fig. 1. Survivorship of lice after body cavity inoculation with *Pasteurella tularensis*. The lines represent (A) normal lice, not punctured, (B) normal lice, punctured, and (C) infected lice.

In order to confirm statistically the difference in survivorship between the infected and control groups, the percentages were transformed to logits and the days to logarithms, since these two metameters appeared to approximate a linear relation most clearly among the several transformations investigated. Straight lines were fitted separately to each group by the method of least squares. An analysis of variance as shown by Bliss & Calhoun (1954, p. 133) was used to determine significant differences among the slopes of the three lines. Heterogeneity among the three slopes was demonstrated by an F value of 37·6 ($P \ll 0{\cdot}001$). The

slopes of the two control groups did not vary significantly from each other ($F = 1{\cdot}2$, $P > 0{\cdot}05$), while each of the control slopes differed significantly from the slope of the infected group at $P < 0{\cdot}01$. These findings clearly confirm the fatal effect of a haemocoele infection of *P. tularensis* in the louse.

Plate counts made of 10 lice on the initial day of infection resulted in maximum readings of 4000, minimum of 90, and an average of 1300 organisms per louse. In 10 lice tested on the fourth day after infection, the maximum was 90×10^6 organisms per louse, the minimum was 13×10^5 organisms, and the average was 36×10^6. Only five lice were used for plate counts on the seventh day after infection, but these showed even more extensive multiplication of the tularemia organisms, the maximum and minimum being 14×10^7 and 5×10^7, respectively, the average being 11×10^7 organisms per louse. To preserve the numbers of the infected colony from depletion by these counts, the 10 lice for the initial day were extra lice above the 150 originally inoculated. The lice on the other 2 days were moribund. No healthy lice were ever removed from the infected colony for this purpose.

Various means besides plate counts were used to demonstrate the infection in the lice. Crude platings of undilute suspensions were made of seven dead or moribund lice on the third day and five such lice on the eighth day. All 12 lice showed confluent growth of *P. tularensis* on the GCBA plates. A Gram stain of smears of body fluids from 10 lice dead on the fourth day showed abundant typical *P. tularensis*.

Serial sections were made of 10 lice dead on the fourth day and of 3 lice, the last members of the colony, on the ninth and tenth days. All 13 lice showed *P. tularensis* scattered throughout the haemocoele. The older lice appeared to have a heavier infection of the body cavity. Although the organisms were predominantly extracellular, occasionally intracellular *P. tularensis* was observed in various tissues of virtually all of these lice. Sporadic infection of the epidermal cells of the oesophagus, tracheae, body wall, and vaginal wall were observed, along with an additional infection of longitudinal and dorso-ventral muscles, and of the accessory gland in one louse. This last case might be attributed to the penetration of the organ by the inoculating pin. Seven of the lice showed infected epidermal cells surrounding the original pin prick through the body wall. The organisms showed abundant intracytoplasmic development similar to that previously described for the midgut epithelial cells. The cells were usually quite swollen and fully packed with *P. tularensis*. Infected areas typically involved only isolated groups of a few cells. An occasional phagocytic blood cell was seen to contain a number of *P. tularensis*. The small number of organisms in these cells would indicate absence of active intracellular multiplication of the bacteria.

Of the original 150 lice in the infected colony, 50 were examined by one means or another and were all found to harbour *P. tularensis*. Serial sections of eight normal lice were inspected in the control group receiving a pin prick but no *P. tularensis*. In none of these lice could tularemia-like organisms be demonstrated.

DISCUSSION

Tularemia infection in the louse. In a previous study on the multiplication of the tularemia organisms in the human body louse (Price, 1956), it was found that lice were initially 100% infected. Following this initial infection, some lice lost their infection and others showed an increase in the number of tularemia organisms. The percentage of uninfected lice increased with passage of time after initial infection until only a very small percentage retained the infection after 35 days.

In an attempt to explain this phenomenon within an infected louse colony, it is necessary to emphasize certain points concerning the infected louse. Histological study revealed *P. tularensis* limited to the lumen of the midgut of some lice as long as 10 days after infection. Apparently the first manifestation of intracellular development of *P. tularensis* may occur as early as the fourth day after infection or as late as the seventeenth day or even later. As long as the infection was limited to the gut, the louse probably was not injured by the infection, but once the *P. tularensis* penetrated the barrier of the gut wall into the haemocoele, death occurred within a week.

It seems unlikely that a louse may be capable of losing its infection once it has become intracellularly infected. With the passage of time after initial infection the percentage of infected lice showing heavy midgut epithelium and haemocoele involvement becomes progressively larger. Were lice becoming uninfected from a heavily infected level, lice exhibiting lower levels of infectivity would be present at this interval after infection. An examination of Table 1 reveals that such is not the case. The high susceptibility of the body louse to *P. tularensis* in the body cavity lends added support to the belief that heavy louse infection ends in death. Heavy epithelial infection was invariably accompanied by cellular distortion and destruction that made the break-through of the bacteria into the body cavity seem imminent. Since the louse lacks any nidi in its midgut, the replacement of these destroyed cells would not be possible (Sikora, 1916). This progressive picture of the infection in infected lice makes it likely that it was the combination of death of infected lice and disinfection by ridding the gut lumen of the organisms that caused the decreasing percentage of infected lice with time after infection. Those lice which on plate counts showed tremendous development of organisms were undoubtedly those in which invasion of the haemocoele had occurred, and failure to detect more of these is attributed to the fact that only healthy-appearing lice were selected for the counts. Lice suffering from this body-cavity type of infection become moribund or abnormal-appearing a day or two before actual death and thus would not be chosen for plate counts. Those infected lice which persisted the longest were those which were perhaps best able to prolong their lumen infection without cellular involvement, and to prolong cellular invasion longest without a breakdown of the gut wall.

The tularemia-infected cell of the body louse closely resembled the infected cells found by Francis (1927) in *Dermacentor andersoni* and *Cimex lectularius*. The tularemia organisms in all three arthropods invaded the haemocoele; however, whereas the principal site of infection in the louse was in the anterior third of the

midgut epithelium, the tick showed infection of the epithelial cells of the rectal sac, lower intestine, intestinal diverticulae, and Malpighian tubules, and the bed-bug showed intracellular invasion only in the posterior portion of the midgut and occasionally in the Malpighian tubules. There was absence of general multiplication in the gut lumen of the ticks but multiplication in the anterior portion of the bed-bug midgut lumen. Salivary glands of all three failed to demonstrate the presence of *P. tularensis*.

Similarities with rickettsial infections. It is interesting to note that, like the rickettsiae reported by Weyer (1954), *P. tularensis* is able to multiply in the louse haemolymph. It should be noted in this connexion that Weyer's findings are unusual since all pathogenic rickettsiae, except for *Rickettsia quintana*, multiply only intracellularly. Growth of many diverse organisms has been established in the body fluids of the louse. The rickettsiae as well as *Bartonella bacilliformis*, *Trypanosoma melophagium*, and the symbiont of the bed-bug have been shown to multiply abundantly there (Weyer, 1953*a*). The relapsing-fever spirochaete and the tularemia organism also must be added to this list. Weyer believed the haemolymph to be a polyvalent substrate enabling these entirely different micro-organisms to thrive luxuriantly. Even the rapid death of the louse caused by *P. tularensis* presents a situation similar to that observed with some of the rickettsiae. The manner in which these organisms caused the death of the lice was obscure, but Weyer (1952) advanced the suggestion that either a toxic substance of the micro-organism or the blocking of the blood cells might be responsible. The latter possibility was suggested by his consistent finding of numerous rickettsiae in the phagocytic blood cells. The absence of heavy infection of the blood cells by *P. tularensis* makes the hypothesis of toxaemia more likely.

The intracellular development of the tularemia organism in some cells of the body wall, tracheae and muscles following body cavity inoculation had its only reported counterpart with *Coxiella burneti* (Weyer, 1953*b*). One instance was reported by Weyer (1950) of development of *Rickettsia mooseri* in the female gonads following probable injury of these during the artificial body cavity inoculation of the louse. The multiplication of *P. tularensis* in both the cells and contents of the accessory gland of a female louse was believed to have occurred in a similar manner.

In the midgut epithelium, the rickettsiae developed only within the cytoplasm of the infected cells. Degenerative evidence was often revealed by ballooning and vacuolization of the cells. No apparent preference was shown for any region of the midgut. Weyer (1953*a*) attributed this special growth ability in the louse stomach to a fundamental character of all true rickettsiae, which can be interpreted as a sign of common origin. *P. tularensis* showed itself capable of invading the midgut epithelium of the louse in a similar manner, although demonstrating a marked preference for the anterior portion of the midgut. The examination of infected lice 35 days after original infection indicated that this relationship in some cases was not too deleterious to the louse involved.

The foregoing comparisons show that infection of the body louse with *P. tularensis* is similar in most respects to that with the rickettsial pathogens. In view of

its Gram-negative, pleomorphic, and minute nature, as well as its intimate relationship with arthropod tissues, *P. tularensis* resembles rickettsiae quite closely. The rickettsiae, however, are incapable of cultivation on artificial media, and in this respect differ from the tularemia organism. The demonstration of similarities of the above organisms in louse infection offers further evidence on the close relationship of *P. tularensis* to the rickettsiae.

SUMMARY

1. *P. tularensis* may be abundant in the midgut lumen, the epithelial cells of the anterior third of the midgut, and the haemolymph of the louse. Multiplication is extracellular in the lumen and the haemolymph and intracytoplasmic in the gut cells.

2. The multiplication of the tularemia organisms in the midgut epithelium eventually leads to the disruption of these cells and the break-through of the organisms into the body cavity. The growth of these organisms in the haemocoele results in the death of the louse in 4–7 days.

3. Lice vary greatly in their susceptibility to infection. Some lice show complete resistance to infection; others are capable of retaining the infection essentially for their normal life span, i.e. 35 days; still others apparently succumb to a rapid increase of the organisms in a relatively brief time after infection.

4. The behaviour of *P. tularensis* within the louse presents interesting similarities to infection of lice with the rickettsiae pathogenic to humans.

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EXPLANATION OF PLATE XVIII

Fig. 1. *Pasteurella tularensis* in lumen of the midgut of a louse 1 day after infection. (× 500.)

Fig. 2. Early infection of midgut epithelium 4 days after infection of a louse. (× 500.)

Fig. 3. Ballooning infected cells in the caecum of a louse 10 days after infection. (× 500.)

Fig. 4. *P. tularensis*, both intracellular and in the midgut lumen, in a louse 35 days after infection. (× 500.)

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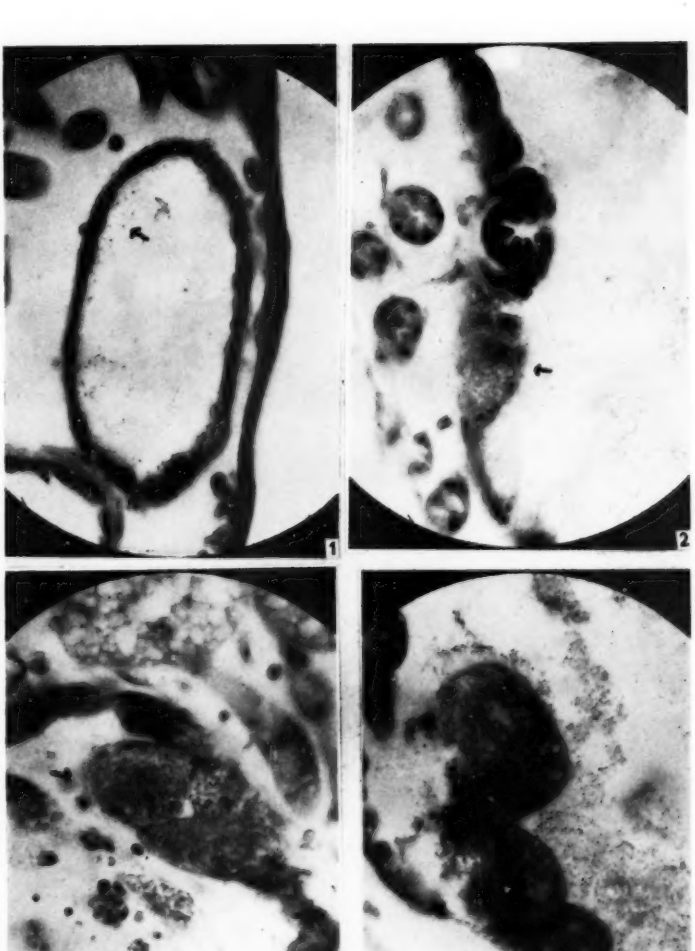

(*Facing p.* 446)

LAMIPPE CONCINNA SP.N., A COPEPOD PARASITIC IN A WEST AFRICAN PENNATULID COELENTERATE*

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(With 13 Figures in the Text)

Copepods belonging to the genus *Lamippe* live in the gastrovascular cavity of several alcyonarian genera. Eighteen species of these copepods are known (Zulueta, 1911), nineteen if the variety *L. rubra decolor* Zulueta 1908 is raised to species rank as suggested by Heegaard (1949). Each species is highly preferential in its choice of a host. Only one species is known to parasitize more than a single species of coelenterate, namely *L. rubicunda* (Olsson, 1869) which lives in two species of *Alcyonium*.

Members of this genus have been reported from the Mediterranean Sea (Italy, southern France), the British Isles, and south-western Sweden (as listed by Zulueta, 1911), and from the 'Ingolf' station 24, 63° 06′ N., 56° 00′ W., in Davis Strait, off the west coast of Greenland (Heegaard, 1949). An unidentified species of *Lamippe* was reported by Versluys (1902*a*, *b*, 1906) from the 'Siboga' station 87, 0° 32′ S., 119° 39′.8 E., in Makassar Straits, between Borneo and Celebes. Joliet (1882) mentioned having seen an apparently unpublished drawing by Lacaze-Duthiers of a *Lamippe* which the latter had found 'en Afrique, dans un Alcyon'. Leigh-Sharpe (1934) found specimens in a very decomposed condition, but referable to *Lamippe*, in alcyonarians from the following 'Siboga' stations: 87 (mentioned above); 156, 0° 29′.2 S., 130° 5′.3 E., between Moluccas and the Vogelkopf of New Guinea; 251, 5° 28′.4 S., 132° 0′.2 E., west of the Aroe Islands; and 297, 10° 39′ S., 123° 40′ E., south of Timor. British species of *Lamippe* were unfortunately omitted from Leigh-Sharpe's (1935) list of copepods from British invertebrates.

Nineteen specimens of the new species of *Lamippe* described below were collected and most kindly sent to me by Mr Alan R. Longhurst of the West African Fisheries Research Institute at Freetown, Sierra Leone.

Lamippe concinna sp.n.

Material studied. Sixteen females, one male, and two specimens whose sex could not be determined, from the gastrovascular cavity of *Virgularia schultzei* Kükenthal dredged during 1955 from a bottom of soft, muddy sand in less than 5 m. depth in the Sierra Leone Estuary off Kissy, near Freetown, Sierra Leone.

Holotype, allotype, and three paratypes deposited in the British Museum (Natural History), five paratypes in the United States National Museum, and the remaining paratypes in the author's collection.

* This study was aided in part by a grant-in-aid from the Society of the Sigma Xi.

Female. Total length (not including the caudal rami, since they are often partly retracted or bent in these preserved specimens) $1{\cdot}248 \times 0{\cdot}282$ mm., based on six well-extended specimens. Segmentation of the body not evident (Fig. 1).

First antenna (Fig. 2) two-jointed, the distal podomere longer and bearing twelve setae. Rostral area between the bases of the first antennae with two minute setae. Second antenna (Fig. 3) three-jointed, the first podomere with a knob on its posterior surface, the last podomere ending in a recurved claw. Mouth region triangular (Fig. 4), with three tooth-like processes behind the mouth and a ridge in front of it. Attached to this ridge two pairs of muscles, apparently used to move the mouth region forward. Other chitinous parts not identifiable.

First leg (Fig. 5) minute, with the basal portion containing a chitinous framework and muscles as indicated in the figure. Outer lobe (exopodite?) bearing two outer, strongly chitinized, uncinate spines and two inner, less chitinized, straighter spines. At each side of the inner of the latter two spines two small subclavate setae. Outer spinule on longest spine not always present.

Second leg (Fig. 6) about the same size as the first, with a similar base and with the outer lobe bearing three spines, the inner two uncinate and strongly chitinized, the outermost spine straight and weakly chitinized. Both pairs of legs without the processes referred to by Zulueta as the endopodites.

Genital openings paired, located at the beginning of the posterior third of the body (Fig. 7), surrounded by delicate chitinized pieces (Fig. 8). Two spermatophores may be attached.

Caudal ramus about 150μ in length including setae, constant in general form (Figs. 9, 10), but sometimes bent or partially retracted (Fig. 7). Each ramus bearing a minute dorsal inner seta and three large terminal setae, one dorsal and two ventral. The terminal setae showing nine to ten delicate chitinous rings and shallowly bifurcate at their tips. In some specimens these setae bent, with the rings slightly telescoped, giving the setae a segmented appearance.

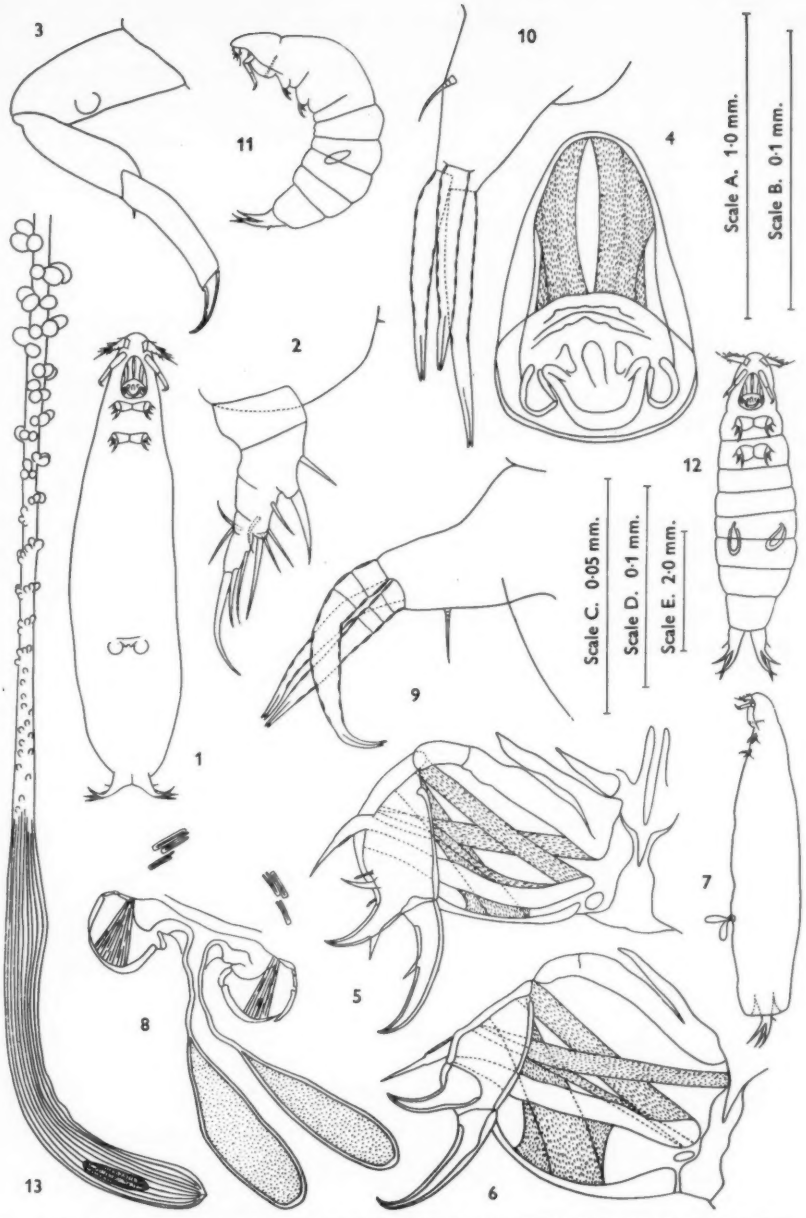
No egg sacs present.

Colour in living specimens reddish.

Male. Total length (not including the caudal rami) $0{\cdot}900 \times 0{\cdot}276$ mm., based on a single specimen (not dissected). This specimen somewhat contracted and showing definite segments (Figs. 11, 12). Six segments posterior to the second legs. Appendages as in the female. Two spermatophores halfway between the second legs and the caudal rami. Each spermatophore about $102 \times 23\mu$, not including the long, sinuous neck. Sex openings not visible on body surface in this specimen mounted in glycerine.

Colour in life reddish.

Remarks. The collector noted in correspondence that 'the copepods were easy to spot in the fresh state, appearing as small reddish dots contained within the cavity of the coelenterate, sometimes in the bulbous base, sometimes in the thin stalk. Those in the stalk appeared to be a younger stage than the ones in the base which were larger and mobile.' The position of one of these copepods is shown in Fig. 13.



Figs. 1–10. *Lamippe concinna* n.sp., female. Fig. 1. Ventral. Fig. 2. First antenna and rostral area. Fig. 3. Second antenna. Fig. 4. Mouth area, ventral (muscles shaded). Fig. 5. Leg 1 (muscles shaded). Fig. 6. Leg 2 (muscles shaded). Fig. 7. Lateral, with two spermatophores attached. Fig. 8. Genital field, with two spermatophores attached. Fig. 9. Caudal ramus, ventral. Fig. 10. Caudal ramus, dorsal. Figs. 11, 12. *L. concinna* n.sp., male. Fig. 11. Lateral. Fig. 12. Ventral, body slightly pressed by cover-glass. Fig. 13. Sketch of *Virgularia* showing *Lamippe concinna* (stippled) of undetermined sex in position within the gastrovascular cavity. All figures were drawn with the aid of a camera lucida. Scale A applies to Figs. 1, 7, 11 and 12; scale B to Figs. 2–4, 9, and 10; scale C to Figs. 5 and 6; scale D to Fig. 8; and scale E to Fig. 13.

The new African species is distinct from all known species of *Lamippe*. Several species in this genus rest upon incomplete descriptions and figures, thus making comparisons of details uncertain or impossible. There are, however, in all cases, sufficient differences in the caudal rami or in the first and second pairs of legs to establish the identity of the species. The new species is unlike *pusilla* Zulueta 1908, *affinis* Zulueta, 1908, *rubicunda* (Olsson, 1869), *aciculifera* Zulueta, 1908, *parva* Zulueta, 1908, *chattoni* Zulueta, 1908, *pallida* Zulueta, 1908, *duthiersi* Joliet, 1882, *brementi* Zulueta, 1910, *papillifera* Zulueta, 1910, *rubra* Bruzelius, 1858, and *proteus* Clarapède, 1867, all of which have five processes or setae on the caudal ramus. It differs from *setigera* Zulueta, 1908, where the caudal ramus has two terminal setae and one dorsal and one ventral seta, from *albida* Zulueta, 1908, which has the same arrangement of setae as in *setigera* but where the setae are short, and from *sympodii* Zulueta, 1910, which has on the caudal ramus one terminal seta, two subterminal smaller setae, and one lateral seta.

The caudal ramus of *pteroidis* Zulueta, 1910, is unknown, but this species differs from *concinna* in having two uncinate setae on the first leg and a single uncinate seta on the second leg. The remaining two species, *olssoni* Zulueta, 1908, and *forbesi* T. Scott, 1901, are rather inadequately known, but it appears from Olsson's (1869) figure 3 that *olssoni* has a caudal ramus without processes but with fine setae (?), and from Scott's (1896) figures 9, 12, and 13, plate IV, that *forbesi* has a caudal ramus with three setae, the first leg with four uncinate setae, and the second leg with three such setae.

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ON *PSILOCHASMUS INDICUS*, SP.N. (FAMILY PSILOSTOMIDAE ODHNER, 1913)

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(With 1 Figure in the Text)

The genus *Psilochasmus* was created by Lühe (1909) with *P. oxyurus* (Creplin, 1825) as its type species. This species was redescribed by Odhner (1913), and in the same year Skrjabin (1913) described *P. longicirratus*. Travassos (1921) added a third species, *P. agilis*. Since then *P. oxyurus* has been recorded by Stunkard & Dunihue (1931), Baugh (1949) and Singh (1954); and *P. longicirratus* has been recorded by Tubangui (1932), Cannon (1938), Hsü & Chow (1938), Yamaguti (1939) and Inamdar & Bhalerao (1944).

P. lecithosus Otte (1926) has been shown by Baylis (1932) to be identical with *Hypoderaeum conoideum*, while *Psilochasmus japonicus* Ishii (1935) has been mentioned by Yamaguti (1939) as a synonym of *P. longicirratus*. Stunkard & Dunihue (1931) pointed out the synonymy of *P. longicirratus* and *P. oxyurus* on the basis of the varying length of the cirrus sac. Their conclusion was supported by Singh (1954), while Hsü & Chow (1938) maintained that the two species are distinct from each other, without giving any reason for doing so. After reviewing the whole literature the author is convinced that Stunkard & Dunihue were correct in suppressing *P. longicirratus* Skrjabin (1913). For the reasons given below *P. agilis* Travassos (1921) seems to be a synonym of *P. oxyurus*. Hence the only valid species so far described is *P. oxyurus*, the type species.

In the present paper a second species is described. The material for the present study was obtained from the common Brahminy duck, *Casarca rutila* Bonap (1838). Only one specimen was obtained from the intestine of one of the five ducks examined.

Psilochasmus indicus sp.n

The body of the worm is 6·688 mm. long and 0·912 mm. broad in the region between the two testes. Anteriorly it is rounded and posteriorly it is provided with a spike, characteristic of the genus, measuring 0·32 mm. in length. The spike is continuous with the cuticle of body. The cuticle is smooth. The excretory bladder is Y-shaped.

The mouth is subterminal, surrounded by an oral sucker measuring 0·352 × 0·384 mm. The ventral sucker is 0·768 × 0·640 mm. in size. It is strongly muscular, projecting out of the body, situated in the second fifth of body length.

The oral sucker is followed by a narrow prepharynx, 0·144 mm. long, which opens into a muscular pharynx 0·176 × 0·192 mm. in diameter. The pharynx leads into a long oesophagus, 1·36 mm. long. The oesophagus bifurcates into two intestinal caeca which extend some distance in front of the posterior extremity of the body.

The two testes are tandem in position, with their margins slightly constricted in the middle. These testes are situated in the anterior part of the posterior third of the body. The anterior testis is 0·480 × 0·352 mm. and lies 2·160 mm. behind the

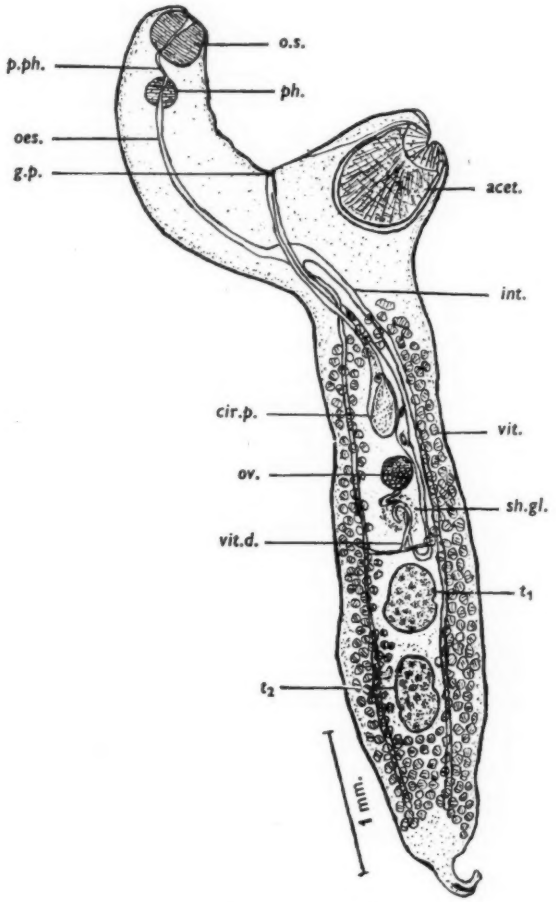

Fig. 1. *Psilochasmus indicus* sp.n. *acet.*, acetabulum; *cir. p.*, cirrus pouch; *g.p.*, genital pore; *int.*, intestine; *oes.*, oesophagus; *o.s.*, oral sucker; *ov.*, ovary; *ph.*, pharynx; *p.ph.*, prepharynx; *sh.gl.*, shell gland; t_1, t_2, testes; *vit.*, vitellaria; *vit.d.*, vitelline duct.

ventral sucker. The posterior testis, 0·496 × 0·288 mm. is 0·112 mm. in front of the anterior testis. Vasa efferentia from the two testes emerge laterally and meet to form a vas deferens at the level of the shell gland mass. The vas deferens opens into a vesicula seminalis, which is enclosed by a cirrus sac. The vesicula seminalis forms a loop anteriorly before opening into a pars prostatica, which leads into a long ductus ejaculatorius. The cirrus sac extends posteriorly some distance in front of the ovary and opens anteriorly into a narrow genital atrium. The genital

pore is situated some distance in front of both the intestinal bifurcation and the ventral sucker.

The ovary 0·224 × 0·240 mm. is spherical, median and is situated in the posterior half of the body. The oviduct emerges from the posterior margin of the ovary and opens into the ootype which is situated immediately behind the ovary. The uterus is very short and contains only 3 yellow ova, 0·080–0·096 × 0·046–0·064 mm.

The vitelline follicles extend from a point 0·400 mm. behind the acetabulum to almost the posterior end of the body. These follicles completely fill the extra caecal region and also overlap the caeca. The vitelline follicles of the two sides meet each other behind the posterior testis and in front of the ovary or behind the ventral sucker. The transverse vitelline ducts arise from the two sides at a level slightly in front of the anterior testis. These ducts unite to form a common vitelline duct which opens into the ootype. A receptaculum seminis could not be seen, but a short Laurer's canal is present.

DISCUSSION

In the genus *Psilochasmus* all the characters seem to be variable. Inamdar & Bhalerao (1944) have discussed the importance of the length of the cirrus sac, the length of the prepharynx and the distribution of the vitelline follicles. They concluded that these characters have no value in differentiating one species of *Psilochasmus* from another. The position of the ovary also varies from a pre-equatorial to a post-equatorial situation. The ratio between the sizes of the two suckers differs from 2:3 to 1:2. It therefore becomes difficult to distinguish the two species of the genus and to recognize the correct position of *P. longicirratus*. This is evident from the fact that, even after Stunkard & Dunihue (1931) had pointed out the synonymy of *P. longicirratus* and *P. oxyurus*, subsequent authors maintained that they are distinct species. The character of the genital opening seems to be fairly constant in all the previous descriptions, except that its position varies from just in front of the ventral sucker to the level of the intestinal bifurcation; but in no instance has it been described to be so far in front of the intestinal bifurcation as it is in *P. indicus* sp.n. This variation in the position of the genital pore can be attributed to the very small space enclosed by the intestinal bifurcation and the acetabulum. This space can slightly increase or decrease because contractions of body change the position of the genital pore.

Specimens of *P. oxyurus* described by Stunkard & Dunihue (1931, p. 180, fig. 2) and of *P. agilis* Travassos (1921) resemble each other so closely in the general topography of the organs, the shape of the body, the position of the genital pore, the extent of the cirrus sac, the distribution of the vitelline follicles and the lobed nature of the testes that it becomes difficult to distinguish *P. agilis* from *P. oxyurus*. Inamdar & Bhalerao (1944) had also expressed doubt about the validity of *P. agilis*. In the light of the above facts *P. agilis* does not appear to be a valid species and it is therefore abandoned. Thus the only valid species so far described is *P. oxyurus* (Creplin) Lühe, 1909.

P. indicus sp.n. differs from *P. oxyurus* in the position of the genital pore, which

is in front of the intestinal bifurcation and shape of testes. These differences justify the creation of a new species for it.

The discrepancies in the descriptions of *P. oxyurus* (including its synonyms) make it necessary to amend the specific diagnosis of this species.

Amended diagnosis of Psilochasmus oxyurus

Body elongate, 2·27–7·3 mm. long and 0·82–1·8 mm. broad. The posterior end of the body provided with a spike. Cuticle smooth. Ventral sucker larger than the oral. Prepharynx small, pharynx well developed, oesophagus of varying length and the intestinal caeca reaching nearly as far as the posterior end of body. Cirrus pouch extending to the pre-ovarian or post-ovarian level. Genital pore situated at the level of the intestinal bifurcation or behind it but in front of the acetabulum. Testes elongate and lobed. Vitelline follicles present from the vicinity of the posterior end of ventral sucker to posterior end of body. Ovary in front of testes. Uterus short containing few ova.

The Ruddy Shelduck or Brahminy duck, *Tadorna ferruginea* (=*Casarca rutila*) is a new host of the genus, which must be added to the list of hosts given by Singh (1954). Yamaguti & Mitunaga (1943) have reported this *Psilochasmus* also from *Columba livia* in Formosa, a host which Singh did not mention.

SUMMARY

1. *Psilochasmus agilis* Travassos, 1921, is regarded as synonym to *P. oxyurus* Lühe, 1909.

2. *P. indicus* sp.n. has been described.

3. An amended specific diagnosis of *Psilochasmus oxyurus* has been given.

4. Ruddy Shelduck or Brahminy duck, *Tadorna ferruginea* (=*Casarca rutila*) is recorded as a new host of the genus.

The author is deeply indebted to Dr H. R. Mehra for suggesting this study and also for the use of his personal library. He is grateful to Dr Dharam Narain for his encouragement and to Dr M. D. L. Srivastava for providing the necessary facilities in the department.

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THE NYCTERIBIIDAE OF THE ETHIOPIAN REGION AND MADAGASCAR

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(With 129 Figures and 3 Maps in the Text)

Only 10 species of Nycteribiidae have been recorded previously from the Ethiopian Region and 3 further species from Madagascar. The writer recently described the new genus *Dipseliopoda* with three species, a new species of *Eucampsipoda* from Africa and another from Madagascar, (Theodor, 1955), 3 new species of *Basilia* and a new species of *Tripselia* from Africa (Theodor, 1956).

The present paper attempts to summarize the information on the family in the Ethiopian Region, to give some information on distribution and host range, to complete the descriptions of known species and to give descriptions of a number of new species.

The paper is based on collections from the Belgian Congo, South Africa, West and East Africa, altogether containing about 1800 specimens. The writer has to thank the authorities of the British Museum (Nat. Hist.), the Museum of the Belgian Congo, Tervueren, the Chicago Natural History Museum, Dr F. Zumpt, South African Institute for Medical Research, Dr R. F. Lawrence, Natal Museum, Professor E. Lindner, Staatl. Museum für Naturkunde, Stuttgart, Dr V. Aellen, Muséum d'Histoire Naturelle, Geneva, Mr G. S. Thompson, Cambridge, Dr J. Bequaert, Museum of Comparative Zoology, Cambridge, U.S.A., and Dr R. Paulian, Institut des Recherches Scientifiques, Madagascar, for placing their material at his disposal.

Together with the new species described elsewhere and in the present paper, 32 species and subspecies are now known from the Ethiopian Region and 6 from Madagascar. Although the material on which the paper is based contains specimens from nearly all parts of Ethiopian Africa, the material from some areas is rather scanty, particularly from Abyssinia, the northern part of tropical West Africa and the part of Africa between the Belgian Congo and Tanganyika and the Union of South Africa. The present list should therefore not be considered as final, and new species are to be expected, particularly from the areas mentioned.

All known genera, except the Oriental genera *Eremoctenia* and *Archinycteribia*, are represented in the Ethiopian Region, and the genus *Dipseliopoda* is, as far as is known, restricted to Africa.

Definitions of genera and subgenera have been given in a paper on the bat parasites of Palestine (Theodor & Moscona, 1954), and in the part on Nycteribiidae in Lindner, *Die Fliegen der Palaearktischen Region*, 66*a* (Theodor, 1954). Some of the species dealt with in the present paper have been described in the papers mentioned, as they reach the Palaearctic Region in the Middle East. Definitions of genera and

subgenera, and descriptions of species described elsewhere, are given in shortened form and the reader is referred to the original papers for further details.

The measurements of the thorax given are those of the sternal plate. The length is measured in the median line and the width in the widest part posteriorly. The terms long and short applied to the abdominal sclerites refer to the anterior-posterior direction.

The nomenclature of the bats has been given according to Allen, 'A checklist of African mammals', 1939, and according to Ellerman, Morrison-Scott & Hayman, *Southern African Mammals*, 1953. The latter authors have made a number of changes, particularly in the genera *Rhinolophus* and *Miniopterus* as far as the species mentioned here are concerned. The synonymy of these species is therefore given.

Previous name	Current name
Rhinolophus acrotis Heuglin	*R. clivosus* Cretschmar
R. augur Andersen	*R. clivosus augur* Andersen
R. geoffroyii Smith	*R. clivosus augur* Andersen
R. geoffroyii zuluensis Andersen	*R. clivosus zuluensis* Andersen
R. aethiops Peters	*R. fumigatus aethiops* Peters
R. lobatus Peters	*R. landeri lobatus* Peters
Miniopterus fraterculus Thomas	*M. schreibersi natalensis* Smith
M. natalensis Smith	*M. schreibersi natalensis* Smith
M. smitianus Thomas	*M. schreibersi natalensis* Smith
M. natalensis arenarius Heller	*M. schreibersi arenarius* Heller

LIST OF THE NYCTERIBIIDAE OF ETHIOPIAN AFRICA AND MADAGASCAR

1. Genus *Nycteribia* Latr., 1796.

   Subgenus *Nycteribia* Latr., s.str.

   1. *N. schmidlii scotti* Falcoz, 1923. Whole of Ethiopian Africa.
   2. *N. exacuta* n.sp. French Guinea.
   3. *N. capensis* Karaman, 1939. South Africa.
   4. *N. latiterga* n.sp. Kenya.
   5. *N. stylidiopsis* Speiser, 1908. Madagascar.

   Subgenus *Stylidia* Westwood, 1840.

   6. *N. scissa* Speiser, 1901. South Africa, Cameroons (?).
   7. *N. scissa sudanica* n.subsp. Sudan, Abyssinia, Belgian Congo.
   8. *N. scissa rhodesiensis* n.subsp. Rhodesia, Nyasaland.
   9. *N. hoogstraali* n.sp. Belgian Congo, Sudan, Kenya.
   10. *N. integra* Theodor, 1954. Arabia, Egypt, Palestine.
   11. *N. tecta* n.sp. South and East Africa.
   12. *N. inopinata* n.sp. Cameroons.
   13. *N. rotundata* n.sp. Rhodesia.
   14. *N. ovalis* n.sp. Sierra Leone, Tanganyika, South Africa.

2. Genus *Basilia* Mir. Ribeiro, 1903.

   Subgenus *Basilia* s.str.

   1. *B. ansifera* Theodor, 1956. West Africa, Belgian Congo, Kenya, Nyasaland.
   2. *B. robusta* Theodor, 1956. East Africa, Abyssinia to Rhodesia, Angola.
   3. *B. daganiae* Theodor & Moscona, 1954. Cyprus, Egypt, Palestine, Kenya (?).
   4. *B. meridionalis* Theodor, 1956. South Africa.
   5. *B. tenuispina* n.sp. Belgian Congo.

   Subgenus *Paracyclopodia* Scott, 1917.

   6. *B. bouvieri* Falcoz, 1924. West Africa, Sudan, Tanganyika.

7. *B. glabra* n.sp. Belgian Congo.
8. *B. madagascarensis* n.sp. Madagascar.

3. Genus *Tripselia* Scott, 1917.
   1. *T. blainvillii* Leach, 1917. Mauritius, Assumption Islands, Madagascar, Africa between 10° lat. north and south.
   2. *T. aequisetosa* Theodor, 1956. West Africa, Belgian Congo.
   3. *T. echinata* n.sp. Nigeria.

4. Genus *Penicillidia* Kolenati, 1863.
   1. *P. fulvida* Bigot, 1885. Tropical Africa from west to east, South Africa, eastern part.
   2. *P. decipiens* n.sp. Madagascar.
   3. *P. pachymela* Speiser, 1901. Africa between 10° lat. north and south.
   4. *P. leptothrinax* Speiser, 1908. Madagascar.

5. Genus *Eucampsipoda* Kolenati, 1857.
   1. *E. hyrtlii* Kolenati, 1856. Arabia, Egypt, Palestine, Syria.
   2. *E. africanum* Theodor, 1955. Belgian Congo, West, East and South Africa.
   3. *E. madagascarensis* Theodor, 1955. Madagascar.

6. Genus *Dipseliopoda* Theodor, 1955.
   1. *D. biannulata* Oldroyd, 1953. Nigeria, Cameroons, Belgian Congo, Sudan, Uganda, Kenya.
   2. *D. setosa* Theodor, 1955. Kenya, Uganda.
   3. *D. arcuata* Theodor, 1955. Abyssinia, Uganda.

7. Genus *Cyclopodia* Kolenati, 1863.
   1. *C. greeffi* Karsch, 1884. Africa between 20° lat. north and 10° lat. south.
   2. *C. greeffi arabica* Theodor, 1954. South-west Arabia.
   3. *C. dubia* Westwood, 1835. Madagascar.

Key to the genera and subgenera of the Nycteribiidae of the Ethiopian Region and Madagascar

1. Eyes absent. **2**
   Eyes present. **4**

2. Tergal plate 2 of the female abdomen with posterior processes which bear long setae or spines. Legs very long. Tibiae 7 to 8 times as long as wide, shorter in one species (Fig. 82). Genus **Tripselia**

— Tergal plate 2 of the female abdomen with straight or uniformly rounded posterior margin. Legs shorter (Figs. 3, 12, 25). **3**

3. Tibiae short and broad, semicircular, 2·5 times as long as wide. Row of setae at the posterior ventral margin of the thorax continuous. Four tergites on the abdomen of the female (in the Ethiopian species) (Figs. 3, 12, 18).
   Genus **Nycteribia**, Subgenus **Nycteribia**

— Tibiae longer, scalpel-shaped, 4 to 5 times as long as wide. Row of setae at the posterior ventral margin of the thorax reduced to 3–4 setae or a single seta at each side. Only 3 tergites on the abdomen of the female (Figs. 25, 42).
   Genus **Nycteribia**, Subgenus **Stylidia**

4. Eyes single, unpigmented. **5**

— Eyes with two ocelli, pigmented. **6**

5. Medium-sized or large, setose insects (3–5 mm.). Haltere groove cover present. Postspiracular sclerite absent. Mesonotum narrowing posteriorly. Oblique sutures of sternal plate of thorax fused, indistinct. Tibiae with several rows of setae distally. Spines of thoracic ctenidium slender and pointed. Spines of abdominal ctenidium short. Numerous long setae on the dorsum of the abdomen of the male (Figs. 89, 96, 98). Genus **Penicillidia**

— Medium-sized, less setose insects (2–3 mm.). Haltere groove open. Postspiracular sclerite present, with a single seta. Mesonotum widening posteriorly. Oblique sutures open, well marked. Spines of thoracic and abdominal ctenidia long, thick and blunt. Tibiae long, cylindrical, with 2 rings and short setae in the middle. Genus **Eucampsipoda**

6. Eyes with two incompletely separated ocelli on a common pigmented base. Tibiae scalpel-shaped, with 3 rows of setae distally. Thorax of *Nycteribia* type. A row of 7–12 notopleural setae present. Male genitalia of *Nycteribia* type. Genus **Basilia** 7

— Eyes with well-separated ocelli on a pigmented base. Tibiae cylindrical, with well-marked rings and short setae in the middle. Mesonotum widening posteriorly. Only 1–3 notopleural setae. 8

7. Tergite 1 of the female abdomen short. Tergal plate 2 with posterior processes which bear long setae (in the Ethiopian species). Distribution of spiracles normal. Tergites of the male abdomen with small spines at the sides of the surface. Tibiae scalpel-shaped, with 3 rows of long setae in the distal half which reach beyond the tip of the tibia (Figs. 57, 58). Subgenus **Basilia** (**B. bathybothyra** group)*

— Tergal plate 1 and sternite 1 + 2 of the female abdomen very long, covering one-half or two-thirds of the abdomen. Spiracles 2–7 concentrated in the posterior third or half of the abdomen of the female. Tergites of the male abdomen practically bare on the surface. Tibiae slender, tapering gradually in the distal half, with 3 rows of short setae in the middle, but without well-marked rings. The setae of the distal row do not reach beyond the tip of the tibia (Figs. 69, 70, 71). Subgenus **Paracyclopodia**

8. Tibiae with two rings. Head moderately compressed laterally. Palps finger-shaped, thickened at the base. Eyes with partly separated ocelli. Postspiracular sclerite with a single spine (Fig. 113). Genus **Dipseliopoda**

— Tibiae with three rings. Head flattened dorsoventrally. Palps flattened. Eyes with ocelli separated nearly to the base. Postspiracular sclerite without setae. Genus **Cyclopodia**

The above key is to some extent artificial and does not express natural relationships satisfactorily. As pointed out elsewhere (Theodor, 1956), the reduction of eyes has apparently developed independently in different groups. Thus *Eucampsipoda* stands near *Penicillidia* in the key, but is more closely related to *Cyclopodia* and *Dipseliopoda*. *Tripselia* is much more closely related to *Basilia* than to *Nycteribia*. To express natural relationships would make the key more difficult to use and would involve the use of the structure of the genitalia.

The definitions given take into account only the Ethiopian species, particularly in the genus *Basilia* and the subgenus *Nycteribia*, and the key for the genera as well as the following keys are not applicable to species from other faunistic regions.

Genus **Nycteribia** Latr., 1796

Diagnosis. Small or medium-sized species (1·5 to 3 mm.) with laterally compressed head. Eyes absent. Palps with 2 rows of setae ventrally and a long terminal seta. Labella of labium as long as the theca or shorter. Notopleural sutures with lateral plates which bear a row of 6–15 notopleural setae. Thoracic ctenidium with long pointed spines. Tibiae flattened, with long setae distally on the ventral edge. Haltere groove without cover. Male abdomen with normal segmentation. Sternite 5 of the male with or without spines at the posterior margin. Tergites of the female abdomen including the anal segment reduced to

* The males of the subgenera *Basilia* and *Paracyclopodia* are difficult to distinguish.

4 or 5. Postspiracular sclerite narrow with several setae. Abdominal ctenidium consisting of closely standing, flattened spines. Either 2 genital plates in the female, of which the dorsal one bears spines, or only a dorsal plate which may or may not bear spines.

Key to the species of the Ethiopian Region and Madagascar

Females

1. 4 tergites on the abdomen (in the Ethiopian species). Anal segment long. Tibiae semicircular, broad and short, 2·5 times as long as wide. The row of setae at the posterior ventral margin of the thorax is continuous with a very long seta at each side (Figs. 3, 4, 8, 18). Subgenus **Nycteribia** 2

— 3 tergites on the abdomen. Anal segment short or very short. Tibiae longer, scalpel-shaped, 4 to 5 times as long as wide. The row of setae at the posterior ventral margin of the thorax reduced to 3–6 setae or a single seta at each side (Figs. 25, 42). Subgenus **Stylidia** 6

2. Abdominal tergites not covering the whole abdomen. Membranous area of dorsum of abdomen covered with short spines between the tergal plates (Figs. 3, 18). 3

— Abdominal tergites covering the whole abdomen without any spines between them. Tergal plates 3 and 4 semicircular (Figs. 8, 12). 5

3. Small insect (1·8–2 mm.). Short spines between tergal plates 2 and 3 and between 3 and 4. Tergal plate 2 very short. Tergal plate 3 also very short and bare on the surface. Anal segment very narrow, parallel-sided, sharply demarcated from the abdomen. Genital plate semicircular, with long or short setae posteriorly, reaching to about half the length of the anal segment. Madagascar (Fig. 18). **N. stylidiopsis** Speiser

— No short spines between tergal plates 2 and 3. Anal segment broad, nearly as wide at the base as the abdomen. 4

4. Length 2–2·25 mm. Tergal plate 3 about three-quarters as wide as tergal plate 2, surface covered with short spines, 4–5 rows of short spines between tergal plates 3 and 4. Genital plate elliptical, not protruding posteriorly, with a row of short spines near the posterior margin (Fig. 3). **N. schmidlii scotti** Falcoz

— Similar, but tergal plate 3 only half as wide as tergal plate 2. 6–8 rows of short spines between tergal plates 3 and 4. Genital plate rectangular, with more strongly sclerotized anterior margin and 6–8 short, strong setae at the posterior margin (Fig. 4). **N. exacuta** n.sp.

5. Smaller insect (2–2·3 mm.). Tergite 1 with 3–4 rows of moderately long setae posteriorly. Tergal plate 2 very long, covering nearly half the abdomen, with straight posterior margin and a small group of short spines in the middle of the surface. Tergal plate 3 with 6 long setae posteriorly, tergal plate 4 with only 2 long setae. Surface of both plates bare (Fig. 12). **N. latiterga** n.sp.

— Larger insect (2·5 mm.). Tergite 1 with a double row of short setae posteriorly. Tergal plate 2 short, bare on the surface. Tergal plates 3 and 4 both with 8–11 long setae in the marginal row. A small group of short spines on the surface of tergal plate 3, tergal plate 4 bare (Fig. 8). **N. capensis** Karaman

6. Only a single short seta on each side of the posterior ventral margin of the thorax. The row of notopleural setae does not extend anteriorly beyond the base of the mesopleural suture (*scissa* group) (Fig. 23). 7

— Several (3–6) setae on each side of the posterior ventral margin of the thorax. The row of notopleural setae extends anteriorly to halfway between the base of the mesopleural suture and the anterior spiracle. (*Biarticulata* group) (Figs. 37, 41). 8

7. Marginal row of tergite 1 consisting of 6–7 short setae in each half. Marginal row of tergal plate 2 consisting of about 10 spines. Only a single or double row of short spines in each half of the surface of tergal plate 2 along the median line. Tergal

plate 3 large, elliptical, with 2 long and several longer and shorter setae at the posterior margin (Fig. 25).

N. scissa Speiser, **N. scissa sudanica** and **N. scissa rhodesiensis** n.subspp.

— Marginal row of tergite 1 consisting of 8–9 longer setae at each side. Tergal plate 2 with a row of 12–14 spines posteriorly in each half. Median part of the surface of the plate covered with short setae, 20–30 in each half. Tergal plate 3 small, rounded, with 2 long setae and 2 short spines posteriorly. The short spines may be absent (Fig. 34). **N. hoogstraali** n.sp.

8. 6 setae at the posterior ventral margin of the thorax at each side of which one is very long. Long setae at the posterior margin of tergite 1, tergal plates 2 and 3. Abdomen bare between tergal plates 2 and 3. Tergal plate 3 very large, roughly rectangular, bare on the surface, with 4–6 long setae posteriorly. Anal segment relatively long, conical. Arabia, Middle East (Figs. 37, 38). **N. integra** Theodor & Moscona

— Only 3 or 5 shorter setae at each side of the ventral posterior margin of the thorax. Short or very short setae at the posterior margin of tergite 1. Dorsum of abdomen covered with short spines between tergal plates 2 and 3. 9

9. Tergal plate 2 large, twice as long as tergite 1, rounded, with long marginal setae. Tergal plate 3 wide, in the shape of a circle segment, with 2–4 (?) long setae and 2 short spines posteriorly. Surface bare. Anal segment short. Genital plate rectangular, with 4 short hairs posteriorly (Figs. 51, 52). **N. rotundata** n.sp.

— Tergal plate 2 short, about as long as tergite 1. Genital plate without setae, forming a lip which covers the genital opening from above. 10

10. Tergite 1 with a marginal row of 16–18 setae. Tergal plate 2 with only 6–8 short spines in each half of the surface, near the median line. Marginal setae nearly as long as the plate. A row of long setae among the short spines between tergal plates 2 and 3. The short spines are longer than in other species. Tergal plate 3 narrowly elliptical, with 2 very long, 2–4 long setae and some short spines posteriorly. About 6 moderately long setae on the surface. Dorsal genital plate large, semicircular. Ventral genital plate small, triangular (Fig. 42). **N. tecta** n.sp.

— Tergite 1 with a marginal row of about 10 short setae with a gap in the middle. Tergal plate 2 with 10–12 short spines distributed over each median half of the surface. Marginal setae short. No long setae on the dorsum between tergal plates 2 and 3. Tergal plate 3 very large, elliptical, with 2 long setae and some short spines posteriorly and a few short spines on the surface. Dorsal genital plate narrow, rectangular. Ventral plate large, with concave posterior surface and with transverse ridges and scales on the surface (Fig. 53). **N. ovalis** n.sp.

The female of *N. inopinata* is unknown.

Males

1. Tibiae short, semicircular, 2·5 times as long as wide. The row of setae at the posterior ventral margin of the thorax is continuous with a long seta at each side. Subgenus **Nycteribia** 2

— Tibiae longer, scalpel-shaped, 4 to 5 times as long as wide. The row of setae at the posterior ventral margin of the thorax is reduced to 3–6 setae or only a single seta at each side. Subgenus **Stylidia** 6

2. Length 1·8–2 mm. Sternite 5 short, rectangular, its posterior margin straight or slightly concave, with a single row of 6–8 short spines in the middle. The median spines are smaller, the lateral spines are longer. Anal segment short. Aedeagus slightly curved, tapering to a point. Phallobase with long anterior rounded process. Madagascar. (Figs. 21, 22). **N. stylidiopsis** Speiser

— Posterior margin of sternite 5 either strongly convex with a double row of spines or slightly curved with a median process which bears spines. 3

3. Sternite 5 long, strongly convex posteriorly with a double irregular row of 14–16 short, thick, barrel-shaped spines. Anal segment very long, nearly parallel-sided. Tergite 6 very narrow, strip-like, with only moderately long setae, nearly com-

pletely covered by tergite 5. Aedeagus of equal width in the basal two-thirds, tapering sharply to a long, bifid tip which is curved backwards and with large scales at the base of the dorsal membrane (Figs. 1, 2). **N. schmidlii scotti** Falcoz

— Sternite 5 short, rectangular, posterior margin slightly curved with a median process. **4**

4. Tergite 6 strip-like, as in *N. schmidlii scotti*. Posterior margin of sternite 5 with a median pointed process which bears 6–8 short spines. Aedeagus tapering uniformly to a long single point which is curved backwards (Figs. 6, 7). **N. exacuta** n.sp.

— Tergite 6 normal, with a marginal row similar to that of tergite 5. Posterior margin of sternite 5 produced in middle with 4–6 larger and 4–6 smaller spines at the median process. Aedeagus with long, upturned tip which is not bifid. **5**

5. Larger species (2·5 mm.). Tergites 2–6 completely bare on the surface. Abdomen held nearly straight. Tip of aedeagus turned up at right angle (Figs. 10, 11). **N. capensis** Karaman

— Smaller species (2–2·3 mm.). Surface of tergites covered with numerous short hairs, which are smaller and more numerous on the anterior tergites. Tergite 6 bare. Aedeagus with a shorter and less upturned tip than in *N. capensis* and with a bulge on the dorsal surface. Abdomen held strongly curved ventrally (Figs. 15, 16). **N. latiterga** n.sp.

6. Only a single seta at each side of the posterior ventral margin of the thorax. Row of notopleural setae not extending beyond base of mesopleural suture. Femora long. Sternite 5 divided posteriorly by a median indentation, each half bearing a group of spines adjacent to it (Figs. 23, 24, 28). (*scissa* group) **7**

— 3–6 setae at each side of the posterior ventral margin of the thorax. Row of notopleural setae extending to about halfway between the base of the mesopleural suture and the anterior spiracle. Posterior margin of sternite 5 divided by a small indentation or undivided. Femora shorter (Figs. 37, 41, 46, 55). (*biarticulata* group) **8**

7. Posterior row of spines on sternite 5 with very long, long and shorter spines. Median indentation narrow. Parameres with a shorter or longer triangular tip in dorsal view (Figs. 28, 30, 33). **N. scissa** Speiser, **N. scissa sudanica** and **N. scissa rhodesiensis** n.subspp.

— Only short setae, not spines, at the posterior margin of sternite 5. (Some of them at the sides intermediate between a seta and a very long spine.) Median indentation wide. Parameres with narrow, curved tip in dorsal view (Figs. 35, 36). **N. hoogstraali** n.sp.

8. Sternite 5 divided by a median indentation of the posterior margin and with 2 groups of spines as in *N. scissa*. **9**

— Sternite 5 undivided, with a single group of spines. **10**

9. Abdominal tergites with only a few short setae on the surface. Posterior median indentation of sternite 5 rudimentary. Spines of medium length and short spines in the posterior row. Parameres with long triangular tip (Figs. 46, 47). **N. tecta** n.sp.

— Abdominal tergites with numerous short setae, covering about half the surface. Median posterior indentation of sternite 5 large. All spines of the groups near the indentation very short and of about equal length. Parameres with short, triangular distal ends (Figs. 48, 49, 50). **N. inopinata** n.sp.

10. Posterior margin of sternite 5 nearly straight with an irregular double row of about 25 short spines in the middle half. Aedeagus curved with blunt tip. Parameres with a pointed, slightly curved process and with 3–5 longer and shorter setae in the middle of the ventral edge. Arabia, Middle East (Figs. 39, 40). **N. integra** Theodor & Moscona

— Posterior margin of sternite 5 with a median bulge which bears a group of about 45 short spines in 5–6 rows. The group of spines is restricted to the median third of the margin. Aedeagus straight, tapering to a point. Parameres with a long, pointed, pigmented process and 3–4 setae in the basal part of the paramere (Figs. 55, 56). **N. ovalis** n.sp.

Subgenus **Nycteribia** s.str.

The Ethiopian species of the subgenus all belong to the *schmidlii* group with 4 tergal plates. No species of the *pedicularia* group with 3 tergal plates has so far been found in Ethiopian Africa.

1. **Nycteribia schmidlii scotti** Falcoz, 1923

Syn. *Nycteribia scotti* Falcoz, 1923

Nycteribia schmidlii has been fully described previously (Theodor & Moscona, 1954). *N. scotti* was described from 2 males from British East Africa. Examination of extensive material (about 250 specimens) from various localities has shown, however, that the characters on which Falcoz based the description of *N. scotti* are not constant and apparently incorrect in some details. Comparison of specimens from Africa with Palaearctic material shows that there is practically no difference in size. The African specimens are lighter in colour and there are some chaetotactic differences which are mainly quantitative. There are more setae on the tergites of the abdomen of the male in the Palaearctic specimens and the hairs are finer. The armature of teeth at the basis of the dorsal membrane of the aedeagus consists of fewer and larger teeth in the African form, the aedeagus is narrower and its curved tip is shorter. There is some variation in the chaetotactic characters of the African form from various localities.

Falcoz (1923) states that the thoracic ctenidium of *N. scotti* has only 9 spines. Falcoz, to judge from his drawing, worked with alcohol material in which the spines of the ctenidium are often only partly visible. The number of 9 spines would be very unusual for this group and the specimen from Thysville, Belgian Congo, referred to by Falcoz, has been re-examined and found to have 18 spines. Other characters (width of the thorax, angle of the oblique sutures) are so variable that the differences mentioned by Falcoz fall within the range of individual variation. For the above reasons *N. scotti* is considered as a subspecies of *N. schmidlii*.

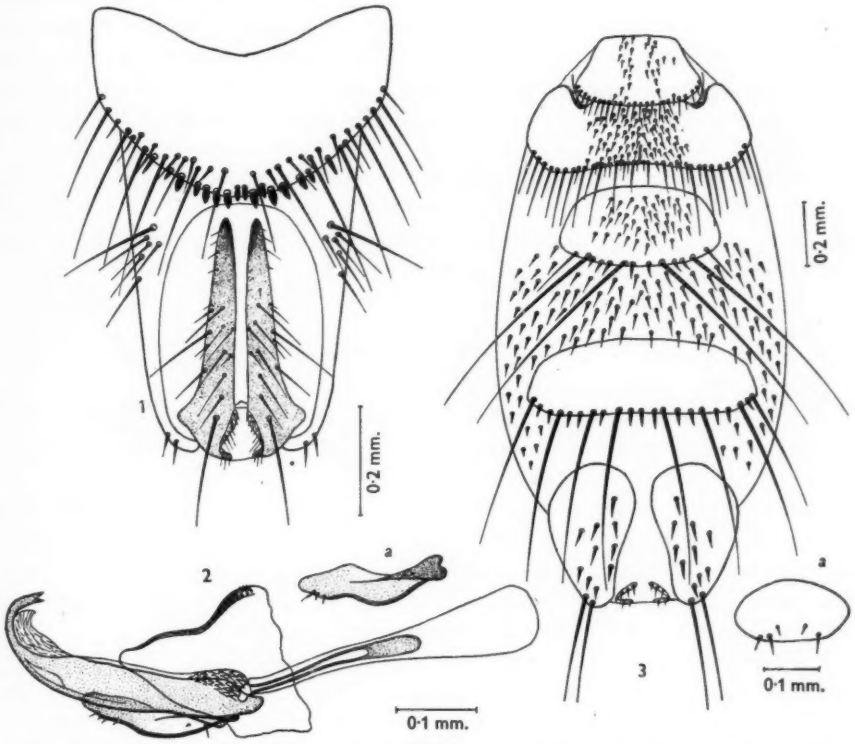
The main characters of the subspecies are as follows:

Male. Tergites 2 and 3 of the abdomen with numerous small hairs in the middle of the surface. Tergite 4 with less and larger hairs on the surface. Tergites 4 and 5 with 2–4 very long setae in the middle of the marginal row. Tergite 6 very narrow, strip-like, often covered by tergite 5 and with only moderately long setae in the marginal row. Anal segment very long, nearly parallel-sided, as long as the 4 preceding tergites. Abdomen held strongly curved ventrally. Sternite 5 (Fig. 1) large, bare, strongly convex posteriorly with an irregular double row of 14–16 short, thick, barrel-shaped spines of different size at the posterior margin.

Genitalia (Fig. 2). Aedeagus with a long, curved, bifid tip which curves backwards. Dorsal membrane of the aedeagus with a few rather large, scale-like teeth at the base. These teeth are smaller and more numerous in *N. schmidlii*. The dorsal membrane terminates distally in two processes.

Female (Fig. 3). Tergal plate 2 with rather short, thin setae at the posterior margin and numerous small hairs on the surface. Tergal plate 3 elliptical, with 4 long setae and short spines posteriorly (6–8 long setae and more spines in

N. schmidlii as a rule). Tergal plate 4 rectangular, nearly as wide as the abdomen, bare on the surface, with 6–8 long setae posteriorly and about 10 short spines between them. (These setae and spines are also more numerous in *N. schmidlii*.) Sternites 5–7 with lateral sclerites. Genital plate elliptical with a row of 4–6 short, widely spaced hairs.



Figs. 1–3. *Nycteribia schmidlii scotti*. 1, male, sternite 5 and genital area. 2, male, genitalia: *a*, paramere, dorsal. 3, female, abdomen, dorsal: *a*, genital plate.

Material examined

West Africa. *Nigeria*: Ondo, *Pipistrellus culex*, 1 ♀ (Boulton). *Cameroons*: Mt Cameroon, *Miniopterus inflatus*, 3 spec. (Boulton); Buea, *M. inflatus*, 1 ♂ (Eisentraut); Ndikiniméhi, *Pipistrellus nanus*, 1 ♂ (Aellen); Nkoétyé Ebolowa, *Miniopterus inflatus*, 8 spec. (Perret).

Belgian Congo. Kakontwe, Katanga, 8 spec. (Leleup); Thysville, *M. inflatus*, *M. minor*, *Hipposideros caffer*, 10 spec. (Bequaert, Fain, Leleup); Yolohafiri cave, Irumu district, 30 spec. (Leleup).

East Africa. *Sudan*: Equatoria province, Torit, *Miniopterus schreibersi arenarius*, 1 ♀; Imurok near Torit, *Miniopterus* sp., 3 spec.; Katire near Torit, *Eptesicus* sp., 1 ♂ (Owen). *Kenya*: Ngong near Mt Elgon, *Miniopterus* sp., 14 spec. (Cade); Mt Elgon, *M. schreibersi arenarius*, 60 spec. (Edwards); Mt Menengai,

6 spec. (Hoogstraal); Kapretwa, Kitale, *M. schreibersi arenarius*, 6 spec. (Hopkins). *N. Rhodesia*: Mweru Lake, 3 spec. (Bedford).

SOUTH AFRICA. *S.W. Africa*: Windhoek, *M. schreibersi natalensis*, 1 ♀ (Jordan). *Cape Province*: Willowmore, *Rhinolophus capensis*, 1 ♀ (Bequaert); Grahamstown, *R. capensis*, *Eptesicus capensis*, 4 spec. (Graham). *Natal*: Pietermaritzburg, *Minopterus schreibersi natalensis*, 37 spec., *Rhinolophus clivosus augur*, 21 spec. (Lawrence), *Pipistrellus nanus*, 1 ♀ (Zumpt). *Transvaal*: Pretoria, *Miniopterus schreibersi natalensis*, 6 spec. (Bequaert); Onderstepoort, Pretoria, *M. schreibersi natalensis*, 6 spec. (Bedford); Makapan caves, Potgietersrust, *Rhinolophus clivosus augur*, 8 spec.; Sterkfontein caves, Kruegersdorp, *Miniopterus schreibersi natalensis*, 1 ♂ (Zumpt).

The distribution thus seems to extend throughout Ethiopian Africa, but no specimens have been examined from the countries west of Nigeria. The main hosts are species of the genus *Miniopterus* and less frequently species of *Rhinolophus*. Only a few specimens were found on species of *Eptesicus* and *Pipistrellus*.

2. **Nycteribia exacuta** n.sp.

Length 2 mm. Colour yellowish brown.

Head compressed laterally, anterior median part of the dorsal surface membranous. 2 setae at the anterior dorsal margin. 6–8 setae on the genae. Labella of the labium as long as the theca.

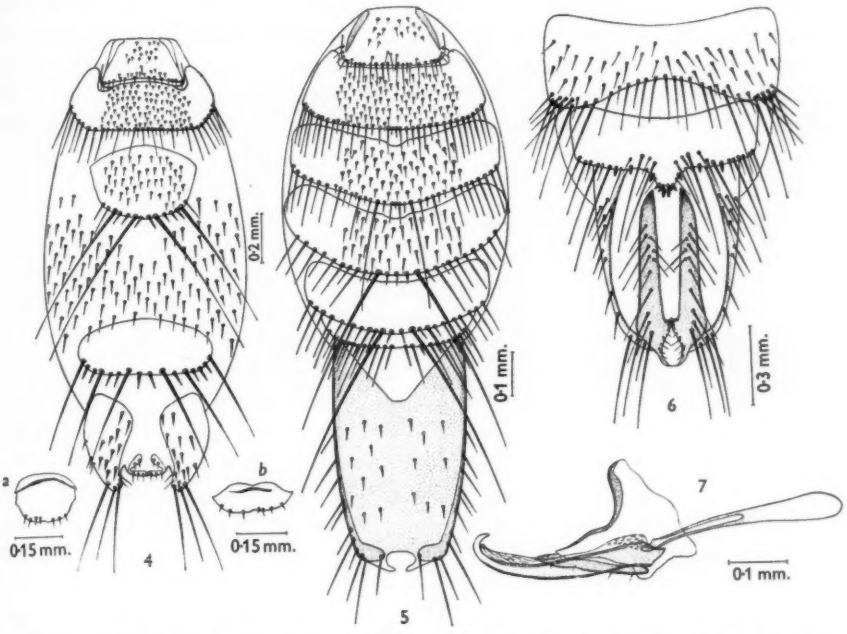
Thorax as long as wide. Thoracic ctenidium with 16–18 long, pointed teeth. Median sternal suture well marked. Angle of oblique sutures about 90°. Posterior margin of the sternal plate with a row of short setae of varying length and 2 very long setae laterally. Mesonotum moderately wide. 6–8 notopleural setae of moderate length. Legs as in other species of the subgenus.

Abdomen, female (Fig. 4). Very much as in *N. schmidlii scotti*. Tergal plate 3 less wide than in *N. schmidlii scotti*, about half as wide as tergal plate 2. Both plates covered with minute spines. Tergal plate 4 wide and short, rectangular, bare on the surface and with a marginal row of 6 long setae alternating with short spines. 6–8 rows of short spines between tergal plates 3 and 4 as against 4–5 rows in *N. schmidlii scotti*. Anal segment as in *N. schmidlii scotti*. Sternite 1 + 2 narrow, rectangular, with a ctenidium of 38–40 teeth (50 in *N. schmidlii scotti*). The marginal setae of the abdominal sternites are less numerous and shorter than in *N. schmidlii scotti*. Genital plate rectangular, wider anteriorly. The posterior margin bears a row of 6–8 short, strong spines and the anterior margin is more strongly sclerotized.

Abdomen, male (Figs. 5, 6). In general, as in *N. schmidlii scotti* but the tergites are shorter. The anal segment is parallel-sided and 1·7 times as long as wide. (Twice as long as wide in *N. schmidlii scotti*.) (Alcohol material.) Sternite 5 is short and rectangular with a median pointed process of the posterior margin which bears 6–8 short spines and there are a few similar spines farther laterally. In one specimen there are 2 larger and 2 smaller spines at the tip of the process.

Genitalia (Fig. 7). Claspers as in *N. schmidlii scotti*. Basal arc rounded with short

anterior process. Phallobase concave dorsally. Aedeagus uniformly tapering to a long point which is curved backwards and not bifid as in *N. schmidlii scotti*. The dorsal membrane of the aedeagus bears small scale-like teeth at the base and ends apically in two processes which are narrower and shorter than in *N. schmidlii scotti*. Parameres parallel-sided, with blunt tip and 4–5 short hairs at the ventral edge.



Figs. 4–7. *Nycteribia exacuta* n.sp. 4, female, abdomen, dorsal: *a*, *b*, dorsal genital plate. 5, male, abdomen, dorsal. 6, male, sternite 5 and genital area. 7, male, genitalia.

N. exacuta is closely related to *N. schmidlii*. It differs from it mainly in the shape of the genital plate in the female and in the form of sternite 5 and the genitalia in the male.

Material examined

French Guinea: Grotte du Marché, Dalaba, *Miniopterus inflatus*, 2 ♂, 2 ♀ (Villiers). Type series, Muséum d'Histoire Naturelle, Geneva.

3. **Nycteribia capensis** Karaman, 1939

Syn. *Nycteribia pedicularia* Latr. apud Speiser, 1901

Karaman (1939) described the species from a single female. The male is here described for the first time.

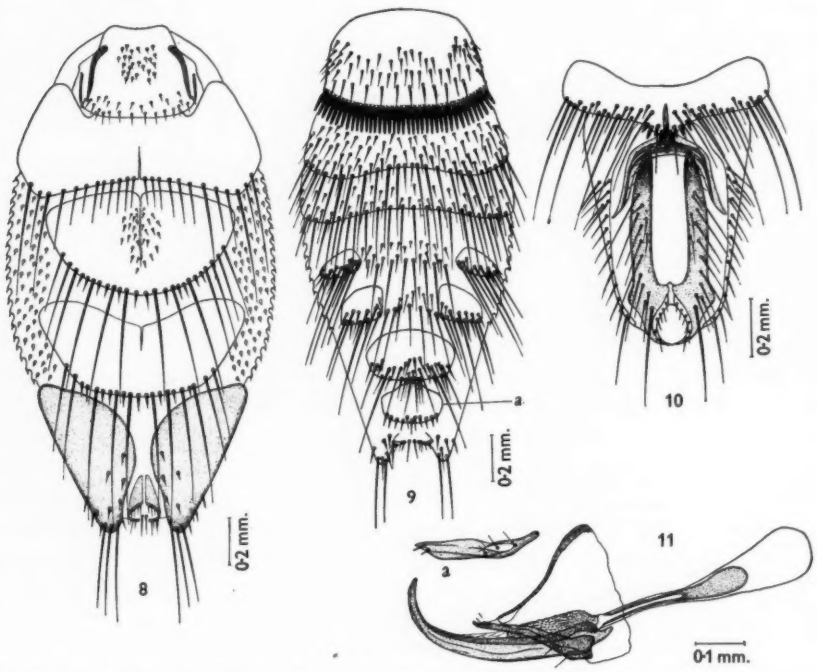
Length 2·5 mm. Colour light brown.

Head moderately compressed laterally, anterior third of dorsal surface

membranous. 2 setae at the anterior dorsal margin. Genae with 6–8 short spines. Labella of labium about as long as the theca.

Thorax wider than long. Length to width = 5:6. Thoracic ctenidium with about 23–25 long, pointed teeth. Median sternal suture well marked, widened in the posterior two-thirds. Oblique sutures forming an angle of 90°. Posterior margin of the sternal plate with a row of shorter and longer setae, of which one at the sides is very long. Dorsal pattern as in other species of the subgenus. A row of 8–10 notopleural setae. Legs as in other species of the subgenus. Tibiae 2·5 times as long as wide.

Abdomen, female (Figs. 8, 9). Tergite 1 with straight posterior margin. A double row of short setae before the margin. Tergal plate 2 wide, triangular, incompletely



Figs. 8–11. *Nycteribia capensis*. 8, female, abdomen, dorsal. 9, female, abdomen, ventral: *a*, genital plate. 10, male, sternite 5 and genital area. 11, male, genitalia: *a*, paramere.

divided in the middle, bare on the surface and with a row of equally spaced setae posteriorly. 3–5 setae at the sides are very long and there are only shorter setae towards the middle. Tergal plate 3 semicircular, with a median fold which begins at the anterior margin and does not reach the posterior margin. This bears a row of 10–12 long setae alternating with short spines. A small group of short hairs in the middle. Tergal plate 4 similar, but undivided, bare on the surface, with a similar marginal row which, however, is restricted to the middle part and has 8–11 long setae and less spines. Anal segment large, conical, bare dorsally, except for

4–5 short spines in the posterior part of the surface in each half and 3–4 long setae at the short anal processes. Pleurae covered with minute, thick spines. Sternite 1+2 rectangular, with a ctenidium of 40–44 long teeth. 3–4 rows of short setae on the surface, some setae of the posterior row longer than the others. Postspiracular sclerite narrow, curved, with 4–5 moderately long setae towards the spiracle. Sternites 3 and 4 with a uniform row of long setae posteriorly and 4–5 rows of short setae on the surface of sternite 3, and 2–3 rows on sternite 4. Sternite 5 with 2 small, elliptical sclerites laterally which bear a double row of long and short setae posteriorly. Between them a row of 5–7 long setae and anterior to these a double row of short setae. Sternite 6 similar but the lateral sclerites are larger and stand more closely together. 5–7 setae between them and a row of 5–6 short setae anterior to them. Sternite 7 elliptical with a marginal row of moderately long setae and a similar premarginal row with 2 long setae laterally. A third row of 3–4 short setae in the middle. Genital plate large, triangular or elliptical with a row of 10–12 moderately long and strong spines at the posterior margin.

Abdomen, male (Fig. 10). Tergites 2–6 completely bare on the surface. The marginal row of tergite 2 consists of moderately long setae. 2–4 longer setae and some short spines in the marginal row of tergite 3. The marginal rows of tergites 4–6 consist of about 6 very long setae alternating with short spines. Anal segment conical, with about 12 short spines in the middle of the dorsal surface and 2–3 long setae posteriorly. Sternite 1+2 as in the female. Sternites 3 and 4 with marginal rows of long and shorter setae and short setae on the surface. Sternite 5 narrow, with concave anterior margin, triangularly produced posteriorly with a double row of about 10 short, thick spines at the apex. The posterior median spines are larger than the anterior and lateral spines. The abdomen is held nearly straight.

Genitalia (Fig. 11). Claspers thick, slightly curved, with blunt, dark tip. A long seta dorsally near the base and a row of long setae in the basal half of the dorsal surface. Basal arc broadly rounded with long anterior process. Phallobase concave dorsally with a group of about 8 minute spines in a double row near the base. Aedeagus long, with a long upturned distal end, tapering to a point. Parameres narrow with a rounded tip which bears 2–3 short hairs at the tip and 2 longer hairs in the basal part. The dorsal membrane of the aedeagus is covered with minute scale-like teeth in the basal half.

Material examined

Natal: Pietermaritzburg, *Myotis* sp., 6 spec. (Zumpt); Pietermaritzburg, *Miniopterus schreibersi natalensis*, 1 ♀, *Myotis tricolor*, 10 spec. (Lawrence). The type was found in Cape Province on *Miniopterus schreibersi*.

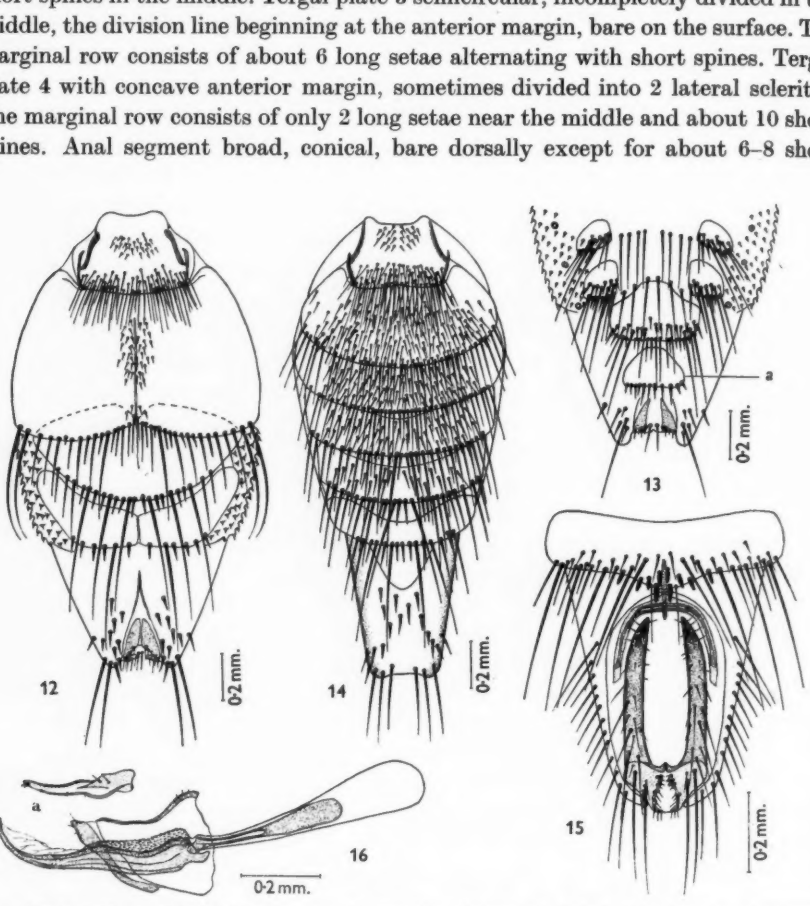
4. **Nycteribia latiterga** n.sp.

Length 2–2·3 mm. Colour light brown.

Head, thorax and *legs* similar to those of *N. capensis*.

Abdomen, female (Figs. 12, 13). Tergite 1 with straight posterior margin and 3–4 rows of moderately long, thin setae near it. Tergal plate 2 very long, covering nearly half the dorsum, rectangular, with a rudimentary median division line. An

elliptical group of small hairs in the middle of the surface. The marginal row consists of 2–3 long setae at each side and of moderately long setae alternating with short spines in the middle. Tergal plate 3 semicircular, incompletely divided in the middle, the division line beginning at the anterior margin, bare on the surface. The marginal row consists of about 6 long setae alternating with short spines. Tergal plate 4 with concave anterior margin, sometimes divided into 2 lateral sclerites. The marginal row consists of only 2 long setae near the middle and about 10 short spines. Anal segment broad, conical, bare dorsally except for about 6–8 short



Figs. 12–16. *Nycteribia latiterga* n.sp. 12, female, abdomen, dorsal. 13, female, abdomen, posterior part, ventral: *a*, genital plate. 14, male, abdomen, dorsal. 15, male, sternite 5 and genital area. 16, male, genitalia: *a*, paramere, dorsal.

spines in the posterior part and several long setae at the anal processes. The great development of the anterior tergites gives the abdomen a characteristic hump-backed appearance in side view in gravid females. Pleurae covered with small spines. The ventral surface resembles that of *N. capensis* except for minor details. The sclerites of sternites 5 and 6 are rather small and protrude from the surface. The posterior row of setae points backwards and the premarginal rows stand vertically. Sternite 7 is triangular and bears a row of 4 long setae and of short spines at the posterior margin. Two of these setae stand at the sides and 2 in the middle. The marginal row points backwards and the premarginal row stands

vertically. Genital plate large, triangular, with a row of 12–15 short spines at the posterior margin.

Abdomen, male (Figs. 14, 15). Held strongly curved ventrally as in *N. schmidlii*. Tergite 1 as in the female. Tergites 2 and 3 with marginal rows of moderately long, thin setae. Nearly the whole surface covered with small hairs. Tergites 4–6 with marginal rows of 4–6 very long setae in the middle, alternating with groups of 2–3 short spines and with shorter setae laterally. The short setae on the surface of tergite 4 are longer and less numerous than on tergites 2 and 3. There is only a double row on the surface of tergite 5 and tergite 6 is bare. Anal segment narrow, parallel-sided, as long as the 3 preceding segments, with short setae on the dorsal surface except in the anterior third. Sternite 5 as in *N. capensis*, with 9–12 short spines in a double row at the apex. The median spines of the posterior row are larger than the others.

Genitalia (Fig. 16). Claspers as in *N. capensis*, but longer and thinner. Basal arc broadly rounded with long anterior process. Phallobase concave dorsally. Aedeagus very long, tapering to a long upturned tip. The aedeagus is less curved than in *N. capensis*, has a dorsal bulge and tapers sharply from about the middle. In *N. capensis* the tapering begins at about two-thirds and is more gradual.

Material examined

Kenya: Mt Menengai, *Miniopterus schreibersi arenarius* and *Myotis tricolor*, 30 spec. (Hoogstraal). Type series, Chicago Museum of Natural History.

N. latiterga is closely related to *N. capensis*, from which it differs mainly in the form of tergal plate 2 and the chaetotaxy of the female abdomen. In the male it differs in the presence of small hairs on the surface of the tergites of the abdomen and in the shape of the aedeagus.

5. **Nycteribia stylidiopsis** Speiser, 1908

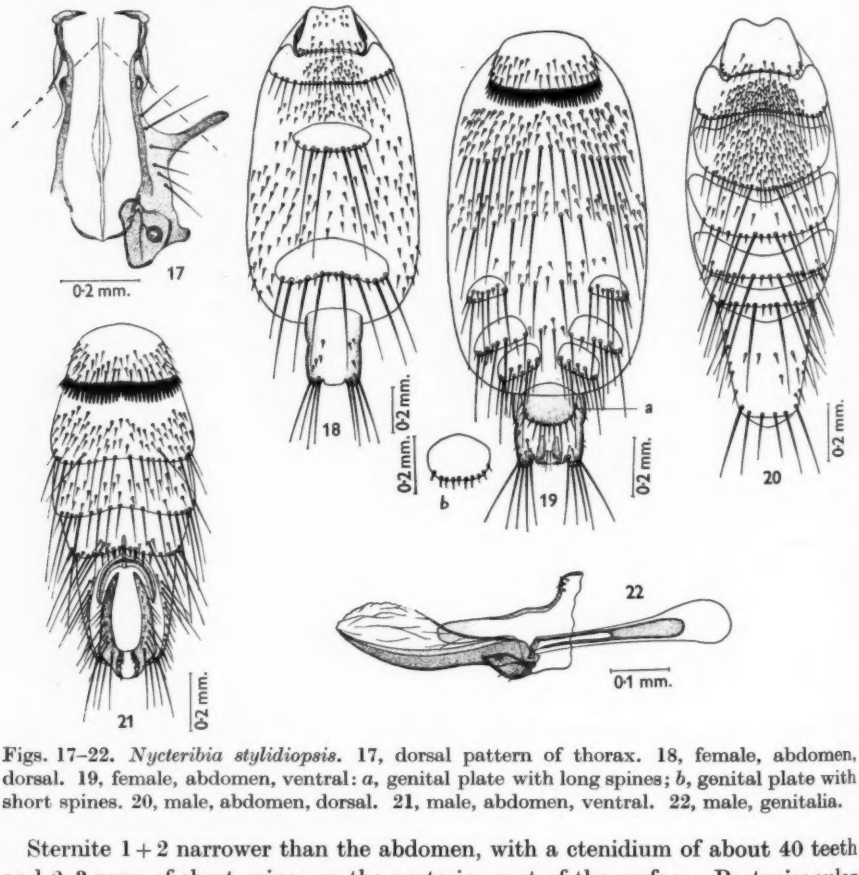
Length 1·8–2·2 mm. Colour yellowish.

Head as in *N. capensis*.

Thorax (Fig. 17) as long as wide. Median sternal suture widened in middle. Oblique sutures forming an angle of 85°. Posterior margin of the sternal plate with a row of moderately long and short setae and 2 very long setae laterally. Thoracic ctenidium with about 20 long, pointed teeth. Mesonotum narrow. A row of 7–8 rather short and thin notopleural setae reaching nearly to the anterior spiracle. Lateral plates of notopleural sutures narrow. Tibiae of the form usual for the subgenus, 2·5 times as long as wide.

Abdomen, female (Figs. 18, 19). Tergite 1 with straight posterior margin and 2–3 rows of short setae near it. Tergal plate 2 very short, with straight posterior margin. The marginal row consists of longer setae laterally and short setae alternating with short spines in the middle. A group of minute hairs in the middle of the surface. Tergal plate 3 small, narrowly elliptical, bare on the surface, with a marginal row of 4 long setae alternating with short spines and 2 shorter setae laterally. Tergal plate 4 wider, narrowly elliptical or with concave hind margin, bare on the surface and with a marginal row of about 8 long setae alternating with

short spines. Dorsum between tergal plates 2 and 3 and between 3 and 4 covered with short spines which are smaller and stand more closely between tergal plates 2 and 3. Anal segment very narrow, parallel-sided, sharply demarcated from the abdomen ('like a beaver's tail', Speiser). Dorsum of the anal segment with a few short spines in 2 irregular longitudinal rows in the posterior lateral part and 4–5 long setae at the anal processes.



Figs. 17–22. *Nycteribia stylidiopsis*. 17, dorsal pattern of thorax. 18, female, abdomen, dorsal. 19, female, abdomen, ventral: *a*, genital plate with long spines; *b*, genital plate with short spines. 20, male, abdomen, dorsal. 21, male, abdomen, ventral. 22, male, genitalia.

Sternite 1 + 2 narrower than the abdomen, with a ctenidium of about 40 teeth and 2–3 rows of short spines on the posterior part of the surface. Postspiracular plate rather broad, with 2–3 short setae near the posterior margin. Sternites 3 and 4 with marginal rows of long setae and short setae on the surface. Sternite 5 with 2 small elliptical sclerites laterally which bear a double row of long and short setae posteriorly and 4–5 setae between them. A double row of short setae anteriorly to them. Sternite 6 similar, but the sclerites are larger, there are only 2 setae between them and a single row of 4–5 short setae anterior to these setae. Sternite 7 is also divided into 2 oblique sclerites with a double row of long and short setae posteriorly. The genital plate lies further posteriorly than in other species, it is semicircular and covers the basal half of the anal segment. It bears a marginal

row of 6–8 moderately long setae in the middle and some shorter ones laterally. In some specimens there are only short spines. Anal sclerite absent, replaced by 2 setae.

Abdomen, male (Figs. 20, 21). Tergites 1 and 2 as in the female but the short hairs on tergite 2 are more numerous. Tergite 3 rather long, with a marginal row of setae of which 2 near the middle are very long and the others grow shorter towards the sides. Surface covered with small spines. Tergites 4–6 narrow, with concave anterior margins and with marginal rows of long setae alternating with short spines. The 4 median setae are very long. A single row of widely spaced, short setae on the surface of tergite 4 or only a few isolated setae. Tergites 5 and 6 bare. Anal segment short, conical, with a transverse row of short spines in the middle of the dorsal surface. Sternites 3 and 4 rather long, with marginal rows of long and short setae. One seta at each side very long. Surface covered with short spines. Sternite 5 short and narrow, with straight or slightly concave posterior margin. A double row of long and short setae at the posterior margin and a group of 4–6 short spines in the middle. The median spines are smaller than the lateral ones. 1 or 2 longer and thinner spines further laterally.

Genitalia (Fig. 22). Claspers short, slightly curved, tapering to a blunt point, covered with setae dorsally which are longer near the base. Basal arc rounded, with moderately long anterior process. Phallobase concave dorsally, prolonged distally into 2 long rounded processes. A group of about 8 minute spines near the base. Aedeagus slightly curved, tapering to a sharp point. Parameres very short, triangular, with a short, sharp point and 3 short hairs near the tip and 2 at the ventral edge.

Material examined

Madagascar: Bemangidi, Fort Dauphin, bat 4491, 5 spec. (Hoogstraal); Andranoboka, Tananariva, Majunga road, 4 spec. (Tipton); Namaroka, Ambohimiriya and Amboanarabi'c caves, *Pipistrellus nanus* and *Myotis goudoti*, 10 spec. (Paulian); Mt d'Ambre, 7 spec. (Paulian).

N. capensis, *N. latiterga* and *N. exacuta* resemble *N. schmidlii* in the presence of a third tergal plate in the female and in the structure of the male genitalia. The presence of a third tergal plate distinguishes *N. schmidlii* from the other Palaearctic species of the subgenus *Nycteribia*. *N. schmidlii* has an isolated position among the Palaearctic species of the subgenus, and one may assume therefore that it is an Ethiopian form which entered the Palaearctic region in the Mediterranean. *N. stylidiopsis* resembles the Oriental and Pacific species *N. parilis*, *N. bakeri* and *N. sarasini* in the arrangement of the tergites of the female abdomen, but differs from them markedly in the structure of the male genitalia.

Subgenus **Stylidia** Westwood, 1840

The subgenus falls into two groups which may be distinguished as follows:

(1) *Biarticulata* group. 3–6 setae, of which one is longer than the others, at each side of the posterior margin of the sternal plate of the thorax (Figs. 37, 41).

The row of notopleural setae extends anteriorly to about halfway between the base of the mesopleural suture and the anterior spiracle (Fig. 37). The anterior notopleural setae are more widely spaced than the posterior ones. Femur 3 shorter than thorax and sternite 1 + 2 together (including the ctenidium). Sternite 5 of the male incompletely divided by a median indentation of the posterior margin and with 2 groups of spines as in the *scissa* group, or undivided, with a single group of spines. Dorsal genital plate of the female large, with a few minute hairs posteriorly, or unarmed and covering the genital opening from above.

(2) *Scissa* group. Only a single short seta at the sides of the posterior margin of the sternal plate of the thorax. The row of notopleural setae does not extend anteriorly beyond the base of the mesopleural suture (Fig. 23). Legs very long. Femur 3 as long as thorax and sternite 1 + 2 together (including ctenidium). Sternite 5 of the male incompletely divided by a deep median indentation of the posterior margin with 2 groups of spines near the indentation. Dorsal genital plate of the female very small. Ventral plate with concave posterior surface and scale-like ridges.

Scissa group

6. **Nycteribia scissa** Speiser, 1901

The species was described by Speiser from a single male. This male was re-examined by Karaman (1939) who added some details to the description. The female is here described for the first time. The description of this species is given in detail. The following descriptions will be shorter and will emphasize mainly the differences from *N. scissa*.

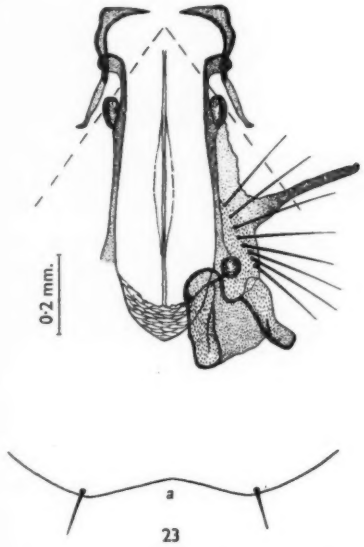
Length 2·5–2·7 mm. Colour light brown.

Head broadly rounded, not laterally compressed, sclerotized up to the anterior margin. This forms an angle which bears 3 setae along each side. 5–6 short setae along the anterior ventral margin of the head and 1 or 2 setae on the genae. Labella of the labium as long as the theca.

Thorax (Fig. 23) as long as wide. Median sternal suture narrow, widened in the middle third. Oblique sutures forming an angle of about 80°. The posterior margin of the sternal plate bears only a single short seta at each lateral bulge. Mesonotum narrow. A row of 7–8 notopleural setae which does not extend anteriorly beyond the base of the mesopleural suture. Legs long. A row of moderately long and strong setae on the ventral surface of the femur proximal to the basal ring. This is best marked on the hind femur. Tibiae long, scalpel-shaped, 4·5–5 times as long as wide with 3 rows of setae in the distal half of the ventral surface. Those of the distal row reach beyond the tip of the tibia. A group of minute spines near the base of the anterior surface of tibia 1 and in the middle of the dorsal surface of tibia 3. Hind femur as long as thorax and sternite 1 + 2 together (including ctenidium). Front femur as long as the thorax (Fig. 24).

Abdomen, female (Figs. 25, 26). Tergite 1 with concave hindmargin which bears a row of about 7 short setae at each side, leaving the concavity bare. The setae grow shorter laterally. Tergal plate 2 incompletely divided in the middle. A row of about 10 short strong setae at the posterior margin in each half. Surface bare

except for a row of 3–5 short spines along the sides of the median line and before the middle of the hindmargin. Tergal plate 3 large, transversely elliptical, with 2 very long setae and several short spines near the hindmargin. Dorsum between tergal plates 2 and 3 covered with minute spines, bare behind tergal plate 3. Anal segment very short, with 2 long setae and some short spines posteriorly at each side and several shorter setae near the base. Pleurae covered with minute spines.



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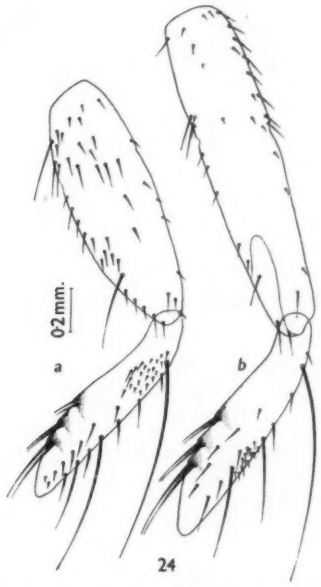

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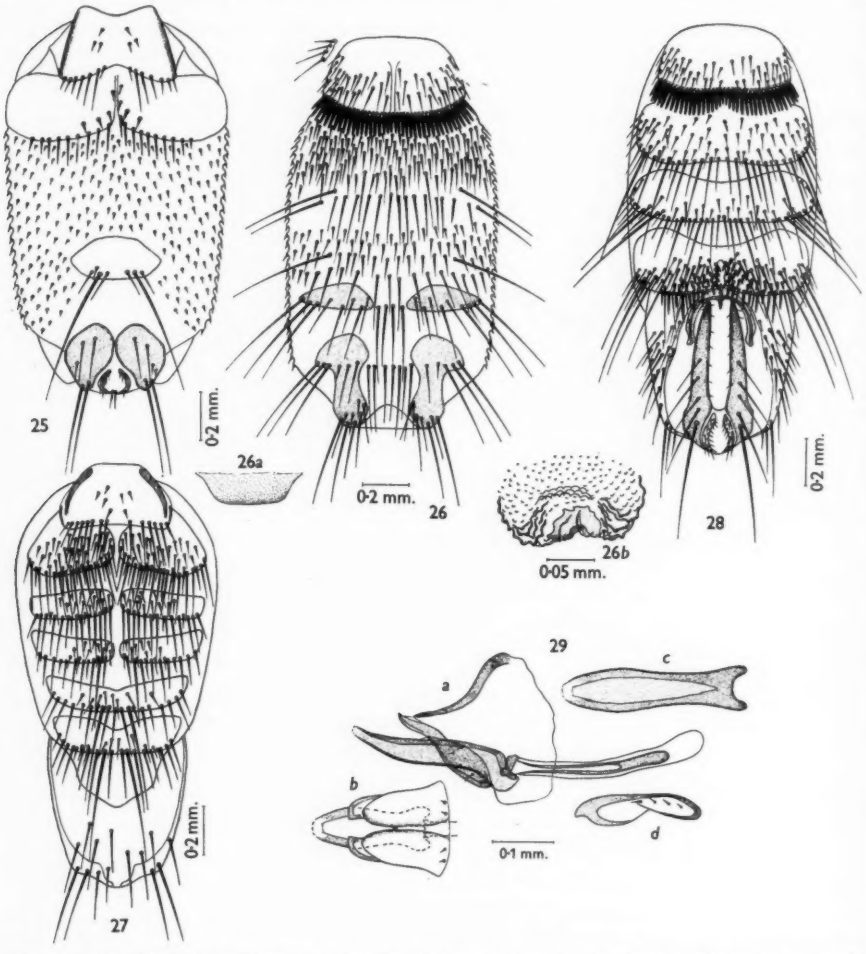
Fig. 23. *Nycteribia scissa*, dorsal pattern of thorax: *a*, ventral posterior margin of thorax.
Fig. 24. *Nycteribia scissa*, legs 1 (*a*) and 3 (*b*).

Sternite 1 + 2 rather short, with a ctenidium of 42–46 teeth and 2–3 rows of short spines on the posterior part of the surface. Postspiracular sclerite narrow, straight, with 4 setae which are longer towards the spiracle. Sternites 3 and 4 with uniform marginal rows of moderately long setae and 4–5 rows of setae on the surface which are longer than in other species. 2 long vertically standing setae in the premarginal and marginal rows of sternite 3, and 2 similar setae in the premarginal row of sternite 4. Sternite 5 with 2 small elliptical lateral sclerites which bear long setae posteriorly and a few short setae on the surface. The rows of long setae continue between the sclerites. The lateral sclerites of sternite 6 are fused with those of sternite 7 which have the form of a longitudinal strip. A row of about 8 setae between the sclerites of sternite 6. The sclerites of sternite 7 bear some long and short setae at the tip. Dorsal genital plate very small, unarmed, rounded, forming a lip. Ventral plate shield-shaped, with concave posterior surface, also without setae and with crinkled or scaly surface (Fig. 26*a*, *b*).

Abdomen, male (Figs. 27, 28). Tergite 1 as in the female but the marginal row

consists of longer and thinner setae. Tergite 2 also similar, but completely divided and shorter and the setae of the marginal row are longer and thinner and the greater part of the surface is covered with short setae. Tergites 3 and 4 divided into lateral sclerites with marginal rows as on tergite 2 and with short setae in the



Figs. 25–29. *Nycteribia scissa*. 25, female, abdomen, dorsal. 26, female, abdomen, ventral: *a*, dorsal; *b*, ventral genital plate. 27, male, abdomen, dorsal. 28, male, abdomen, ventral. 29, male, genitalia: *a*, profile; *b*, phallobase, dorsal; *c*, aedeagus, dorsal; *d*, paramere, dorsal.

median half of the surface. Tergites 5 and 6 undivided, with concave anterior margin and similar marginal rows. 2 long setae in the middle of the row on tergite 5, and 2 very long setae on tergite 6. A few short setae in the middle of the surface on tergites 5 and 6. Anal segment broad, rounded, with deeply concave anterior dorsal margin. Dorsal surface bare except for a single or double transverse row

of short setae in the posterior third and some long setae posteriorly. Sternite 1 + 2 as in the female. Sternites 3 and 4 with marginal rows of long and short setae. The lateral longer than the median setae. Short hairs on the surface. Sternite 5 longer, with concave anterior margin, incompletely divided by a narrow, deep indentation in the middle of the posterior margin. 2 groups of spines adjacent to this indentation which consists of about 4 rows towards the middle and 2–3 rows laterally. The spines of the posterior margin are short near the indentation and become longer laterally. 2–3 spines at the sides are very long. There are some setae between the spines. The spines of the anterior rows are all short and of about equal length. A row of long and short setae lateral to the groups of spines at the posterior margin. A double row of long and short vertically standing setae anterior to the group of spines.

Genitalia (Fig. 29). Basal arc broad, rounded, without anterior process. A short posterior process projecting into the posterior concavity. Claspers thick, nearly straight, tapering to a blunt dark tip. A long seta dorsally near the base and a row of 3–4 setae on the dorsal surface which become gradually shorter towards the middle. Phallobase short, conical, divided by a median dorsal slit. 2 groups of several minute hairs near the base. Aedeagus slightly curved in S-shape in side view, tapering to a blunt point, very wide in dorsal view. Parameres short, with rounded base which bears 4–5 short setae in the basal part and with a broad apical end, tapering to a short curved point in dorsal view. Apodeme rather short with a narrow end-plate.

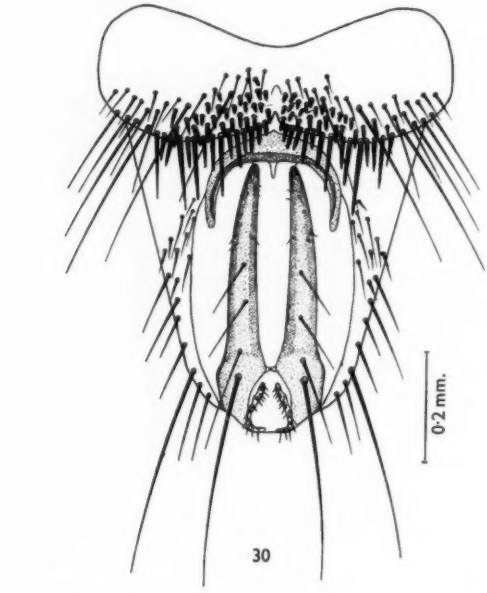
Some specimens from the Cameroons (Tibati, 1 ♂, 1 ♀; Karba Manga, 1 ♂, on *Rhinolophus foxi*) resemble *N. scissa* in all essential characters but differ in some chaetotactic details. There are 2–3 rows of short setae along the median line of tergal plate 2 of the female and the shape of the parameres of the male is slightly different. Two specimens from Lokvi, Sudan, resemble the above specimens from the Cameroons in the female, but differ in the male. The setae on the tergites are less numerous and the spines at the posterior margin of sternite 5 are shorter. More material of these forms will have to be examined in order to determine whether these differences from the South African form are constant so that the creation of a subspecies would be justified.

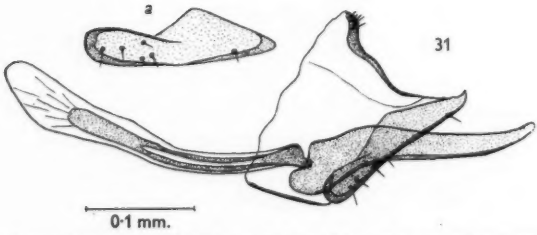
Material examined

Transvaal: Sterkfontein caves, Kruegersdorp, *Rhinolophus clivosus augur*, 10 spec. (Zumpt); Makapan caves, Potgietersrust, *R. clivosus augur*, 10 spec. (Zumpt); Potchefstrom, *R. clivosus augur*, 1 ♂ (De Kaap); *R. clivosus augur*, 2 spec. (Rendall); Pretoria, 2 spec. (Bedford). *S.W. Africa*: Okohandje, *Nycteris capensis*, *Hipposideros caffer* or *Rhinolophus eloquens*, 1 ♂ (Hoesch); Waterberg, *R. clivosus augur*, 1 ♂ (Hoesch). *Natal*: Pietermaritzburg district, *R. clivosus augur*, *R. clivosus zuluensis*, 12 spec. (Lawrence); Ngoya Forest, Zululand, *Rhinolophus* sp., 1 ♂ (Lawrence); Natal, *R. clivosus augur*, 1 ♀ (Wroughton). *Cape Province*: Willowmore, *R. capensis*, 1 ♂ (Bequaert); Knysna, *R. clivosus*, 1 ♀ (Zumpt).

7. **Nycteribia scissa sudanica** n.subsp.

The abdominal ctenidium consists of only 36 teeth in both sexes. The male differs from *N. scissa* in the smaller number of setae on the surface of the posterior abdominal tergites and in the group of spines on sternite 5 (Fig. 30). The posterior row consists of short spines near the median indentation and some long and 3–4 very long spines laterally. There are about 40 spines in each half as against about 30 in *N. scissa*. The shape of the paramere is also different with a triangular tip and the aedeagus is shorter (Fig. 31). The female has 6–8 short setae in a double row near the median line of tergal plate 2.





Figs. 30, 31. *Nycteribia scissa sudanica* n.subsp. 30, male, sternite 5 and genital area. 31, male, genitalia: *a*, paramere.

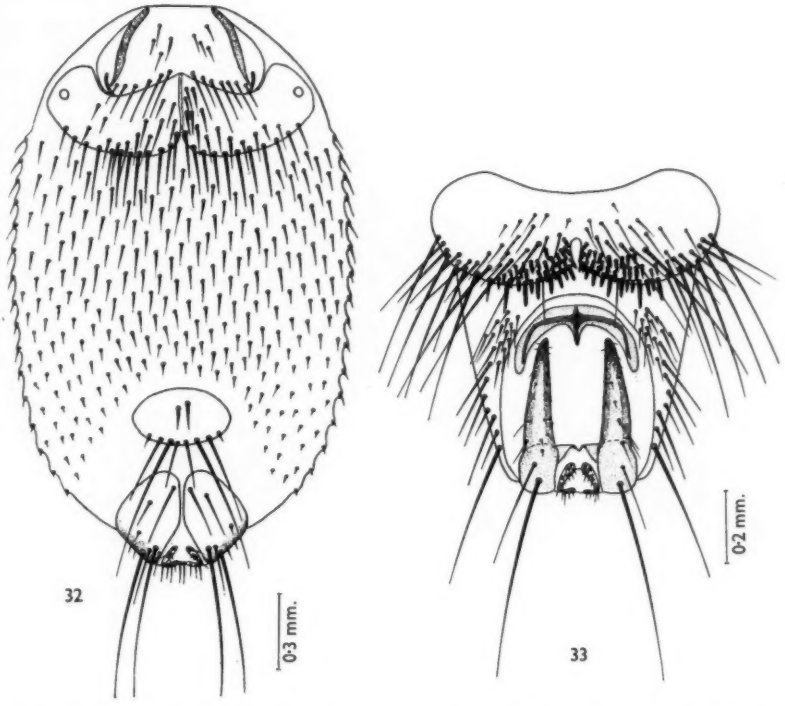
Material examined

Abyssinia: Lake Zwai, 1 ♂ (Jannusch & Goetz). Type. *Sudan*: Lifi near Torit, *Rhinolophus* sp., no. 1901, 1 ♀; Lokwi near Torit, *R. fumigatus*, 4 spec. (Owen). *Belgian Congo*: Mitwaba, *Hipposideros caffer*, 1 ♂.

8. **Nycteribia scissa rhodesiensis** n.subsp.

Length 3 mm. Only 5–7 notopleural setae.

Abdomen, female (Fig. 32). The posterior margin of tergite 1 is deeply concave. The marginal setae of tergite 1 and tergal plate 2 are much longer than in *N. scissa* and there are 2–3 rows of short setae on the surface of tergal plate 2 near the median line and some anterior to the marginal row. Tergal plate 3 as in *N. scissa*, but more regularly elliptical and with some short spines on the surface. The short spines of the dorsum are longer than in *N. scissa* and there are 3 or 4 long setae behind tergite 2 in some specimens.



Figs. 32, 33. *Nycteribia scissa rhodesiensis* n.subsp. 32, female, abdomen, dorsal. 33, male, sternite 5 and genital area.

Abdomen, male (Fig. 33). Tergites 4 and 5 are nearly bare on the surface except for 1–3 short setae in the middle. The group of spines on sternite 5 as in *N. scissa*, but there are only short spines and spines of medium length in the posterior row. The claspers are shorter and thicker than in *N. scissa* and the setae on the dorsal surface of the claspers are much shorter and thinner than in *N. scissa*.

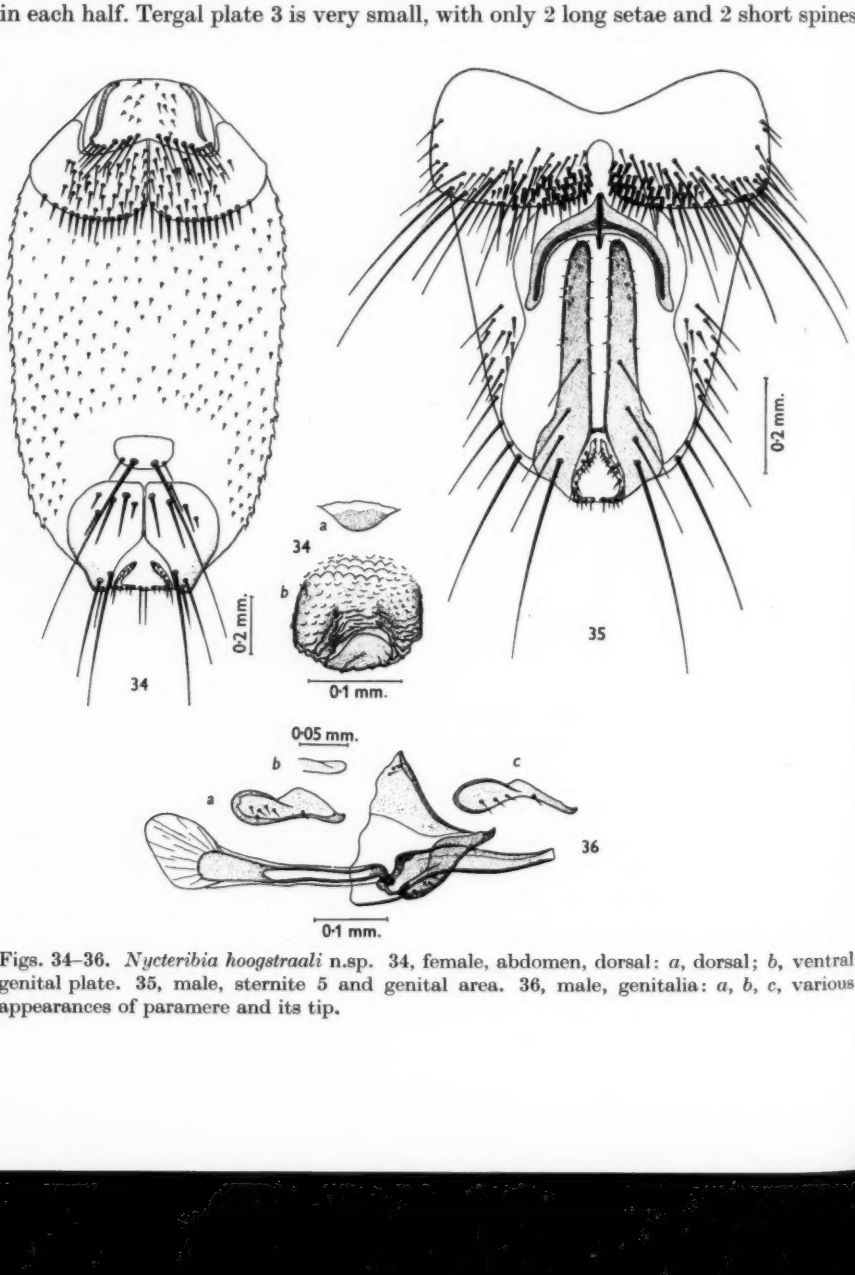
Material examined

Rhodesia: Mazoe, *Rhinolophus hildebrandti*, 2 ♀ (paratypes) (Darling); Mareppa mine, Gwanda, *Nycteris capensis*, 1 ♀ (Carruthers) (type). *Nyasaland*: Chikonje, Ruo, 1 ♂ (Wood). *S. Rhodesia*: Odzi district, *R. darlingi*, 1 ♀ (Miller).

9. **Nycteribia hoogstraali** n.sp.

Size, head, thorax and legs as in *N. scissa*.

Abdomen, female (Fig. 34). The marginal setae of tergite 1 are longer than in *N. scissa* and 8–10 in number. Tergal plate 2 with 12–13 marginal setae in each half. The whole median half or more of the surface is covered with short setae, 20–30 in each half. Tergal plate 3 is very small, with only 2 long setae and 2 short spines



Figs. 34–36. *Nycteribia hoogstraali* n.sp. 34, female, abdomen, dorsal: *a*, dorsal; *b*, ventral genital plate. 35, male, sternite 5 and genital area. 36, male, genitalia: *a*, *b*, *c*, various appearances of paramere and its tip.

lateral to them. These short spines are absent in some specimens. Genital plates similar to those of *N. scissa* (Fig. 34*a*, *b*).

Abdomen, male (Fig. 35). The posterior row on sternite 5 consists mainly of short setae and thin, long spines. There are 2–3 very long spines, intermediate between spines and setae at the sides. The median indentation is much wider than in *N. scissa*. The spines of the anterior rows are short, but longer than in *N. scissa*. The parameres are much shorter than the aedeagus and have a narrow, curved tip (Fig. 36).

Material examined

Belgian Congo: Kakontwe, Katanga, 2 spec.; Lubudi caves, Katanga, 3 spec. (Leleup); Kivu, Rutshuru, 1 ♀ (Luja). *Sudan*: Equatoria province, Katire near Torit and Torit district, *Rhinolophus eloquens*, 28 spec. Type series (Hoogstraal, Owen).

N. hoogstraali and the subspecies *rhodesiensis* and *sudanica* are closely related to *N. scissa*. *N. hoogstraali* is considered as a species as it occurs in the same area as *N. scissa sudanica*. The differences are constant in a relatively large series and are much more marked than in the other 2 forms, where the differences are mainly of degree. As there is very little material of these 2 forms available, the variability of the various characters cannot be determined. They are provisionally considered as subspecies until more material is available.

Biarticulata group

10. **Nycteribia integra** Theodor & Moscona, 1954

This species has been fully described in previous papers (Theodor & Moscona, 1954; Theodor, 1954). The main characters are as follows:

Size 2·5 mm. Colour yellowish brown.

Thorax (Fig. 37). Angle of oblique sutures 80°. 9 notopleural setae. There are 6 setae at each side of the posterior ventral margin of the thorax of which one is very long.

Abdomen, female (Fig. 38). Tergite 1 and tergal plates 2 and 3 with long setae posteriorly. Tergal plate 2 large, rounded, with a double row of short setae near the median line. Abdomen between tergal plates 2 and 3 bare. Tergal plate 3 large, roughly rectangular, bare on the surface and with 4–6 long setae and some shorter ones posteriorly. Anal segment rather long, conical. Sternite 1 + 2 with a ctenidium of 50–55 teeth. Dorsal genital plate shield-shaped, with a few minute hairs posteriorly.

Abdomen, male (Fig. 39). Tergites undivided, marginal rows consisting of moderately long setae and 2 long setae in the middle of the rows of tergites 5 and 6. About 10 short hairs in the middle of the surface of tergite 3 and a few on tergites 4 and 5. Sternite 5 with straight or slightly convex undivided posterior margin which bears a double row of about 25 short spines.

Genitalia (Fig. 40). Aedeagus curved, tapering to a blunt tip. Parameres fused with the phallobase, with a long, curved, pointed process, and 3–5 setae in the middle of the ventral margin.

N. integra is common in Egypt on species of *Rhinolophus* and rare in Palestine. It enters the Ethiopian Region in south-west Arabia (Yemen) and will probably be found in East Africa. Its hosts are mainly *Rhinolophus blasii, R. clivosus* and more rarely *Rhinopoma hardwickei cystops* and *Hipposideros caffer*.

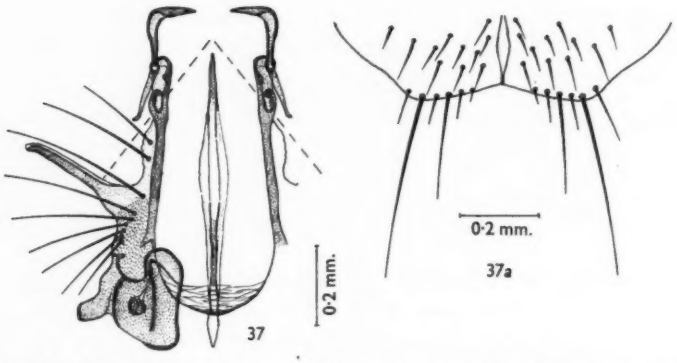


Fig. 37. *Nycteribia integra*, dorsal pattern of thorax: *a*, ventral posterior margin of thorax.

11. **Nycteribia tecta** n.sp.

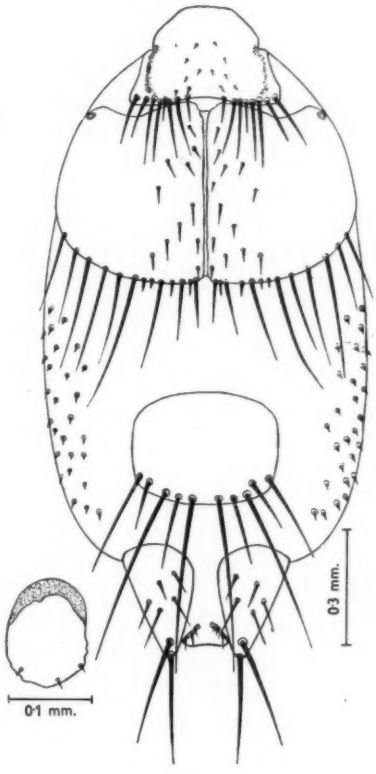
Length 2·2–2·5 mm.

Head as in *N. scissa*. Labella of labium as long as the theca.

Thorax as long as wide. Median sternal suture narrow, widened in the middle third. Angle of oblique sutures about 80°. A group of 3 setae at each side of the posterior margin of the sternal plate of the thorax (Fig. 41). The middle seta of each group is the longest. The row of 9–10 notopleural setae reaches to about halfway between the base of the mesopleural suture and the anterior spiracle. The anterior setae are more widely spaced than the posterior ones. Legs as in *N. scissa*, but shorter. Femur 3 shorter than thorax and sternite 1 + 2 together.

Abdomen, female (Figs. 42, 43). Tergite 1 as in *N. scissa*, but the gap in the middle of the marginal row is smaller. Tergal plate 2 with a marginal row of about 10 rather long setae in each half. A double row of short setae near the median line. 2–3 setae extend laterally in front of the marginal row. Tergal plate 3 transversely elliptical with 4 very long and 4 shorter setae, 2 of which stand between the long setae. 6 short setae in an irregular transverse row on the surface of the sclerite. A row of about 8 moderately long setae halfway between tergal plates 2 and 3. Dorsum of abdomen covered with short spines which are much longer than in *N. scissa* in the anterior two-thirds and very short in the posterior third of the dorsum. Anal segment short with 3 long setae posteriorly on each side and several shorter setae near the base. Sternite 1 + 2 broad, with a ctenidium of 48 teeth. Short setae in 4 rows at the sides and 2–3 rows in the middle of the surface. Sternite 3 with a marginal row of long setae of which one near the sides is very long. Surface densely covered with setae which are about two-thirds as long as the marginal setae. 4 long setae in the premarginal row and 2 in the marginal row at the sides. Sternite 4 similar but the setae on the surface are shorter and there are only 2 long

setae in the premarginal row towards the sides. Sternite 5 divided into narrow, elliptical sclerites with 3 long setae at the posterior margin and a transverse row of 6 shorter setae on the surface. 3–4 setae between the sclerites. Sternite 6



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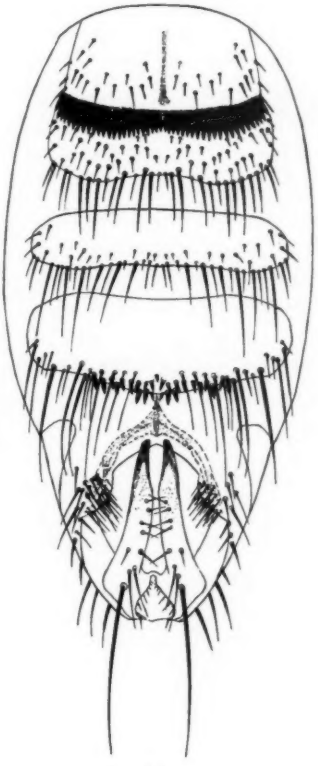

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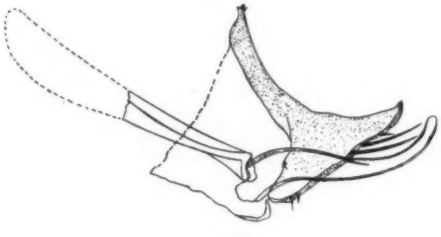
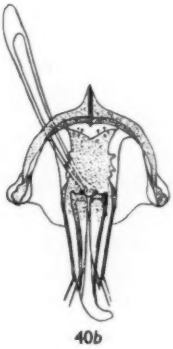


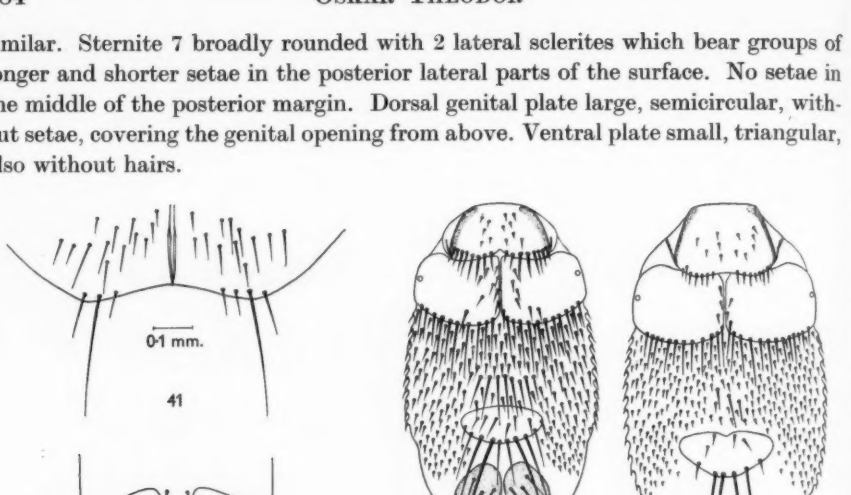
40a



40b

Figs. 38–40. *Nycteribia integra*. 38, female, abdomen, dorsal. 39, male, abdomen, ventral. 40, male, genitalia: *a*, profile; *b*, dorsal.

similar. Sternite 7 broadly rounded with 2 lateral sclerites which bear groups of longer and shorter setae in the posterior lateral parts of the surface. No setae in the middle of the posterior margin. Dorsal genital plate large, semicircular, without setae, covering the genital opening from above. Ventral plate small, triangular, also without hairs.



Figs. 41–47. *Nycteribia tecta* n.sp. 41, posterior ventral margin of thorax. 42, female, abdomen, dorsal: *a*, dorsal; *b*, ventral genital plate. 43, female, abdomen, posterior part, ventral. 44, (?) subsp. female, abdomen, dorsal. 45, (?) subsp. female, abdomen, posterior part, ventral. 46, male, sternite 5 and genital area. 47, male, genitalia: *a*, paramere; *b*, aedeagus, dorsal; *c*, *d*, same of (?) subsp.

The above is the description of the type female from Kenya. A specimen from Southern Rhodesia and another from Pretoria, South Africa, resemble it closely. but differ in some characters (Figs. 44, 45). The labella of the labium are shorter than the theca. The marginal setae of tergite 1 are shorter. There is only a single line of short setae along the median line of tergal plate 2. The short spines of the dorsum are shorter than in the type form and there are only 3–4 setae halfway between tergal plates 2 and 3. The arrangement of the marginal setae on tergal plate 3 is different. There are only 6 setae in the Rhodesian specimen, 2 in the

middle being the longest and there are 4 short spines near the anterior margin of the sclerite. In the specimen from Pretoria there are 4 long setae in the middle and 2 short spines laterally. There is only a single short spine near the base of the anal segment in the Rhodesian specimen and 2–3 in the specimen from Pretoria. Sternite 7 is fused with the sclerites of sternite 6 to form an U-shaped sclerite as in *N. scissa*. This is not so in the type specimen. These specimens will possibly prove to be a subspecies of *N. tecta*.

Abdomen, male (Fig. 46). Tergite 1 as in the female. Tergites 2 and 3 incompletely divided in the middle with marginal rows of moderately long setae. There are 8–10 short setae on the surface in each half near the median line. Tergites 4–6 undivided, with a group of 6–8 setae on tergite 4 and 1–2 or none on tergites 5 and 6. 2 long setae in the middle of the marginal row of tergites 5 and 6. The lateral setae of the marginal rows are very short. Anal segment short and broad, rounded, with 2–3 rows of short setae in the posterior part of the dorsum and some long setae posteriorly. Ventral surface as in *N. scissa*. The posterior median indentation of sternite 5 is very small. The arrangement of the spines resembles that in *N. scissa*, but the posterior row consists of only 3–4 spines of medium length, some near the indentation, and short spines, the very long spines of *N. scissa* being absent.

Genitalia (Fig. 47*a*, *b*). As in *N. scissa*, but the aedeagus is more pointed in dorsal view and the parameres are only slightly shorter than the aedeagus with a rather narrow, pointed distal end and with a row of 6–7 short spines in the basal part which reaches to near the tip.

A male from Southern Rhodesia, from the same locality as one of the females mentioned above as possibly a subspecies of *N. tecta*, perhaps belongs to the same form. It resembles the male of *N. tecta* in most characters and the female from Rhodesia in the shorter labella of the labium. It differs from the male of *N. tecta* in the smaller number of short setae on the surface of the tergites. There are only 5–6 setae on tergites 2 and 3 and 3–4 on tergites 5 and 6. The anal segment is broadly rounded with relatively short setae posteriorly. The groups of spines on sternite 5 consist of 4 rows. Those of the posterior row are not longer than those of the anterior rows except for 2 slightly longer spines at the sides. The parameres are rather short and broad, about two-thirds of the length of the aedeagus (Fig. 47*c*, *d*).

Material examined

Kenya: Ngong near Mt Elgon, *Miniopterus* sp., 1 ♀, type (Cade), Chicago Nat. Hist. Museum; *Uganda*: Kisolo, Virunga Volcano, *Rhinolophus deckeni*, 3 ♂ (Heller), paratypes. *Sudan*: Katire near Torit, Equatoria province, *Eptesicus* sp., 1 ♂ (Owen); Lake Tanganyika, Ujiji, *Rhinolophus* sp. 1 ♂, 1 ♀.

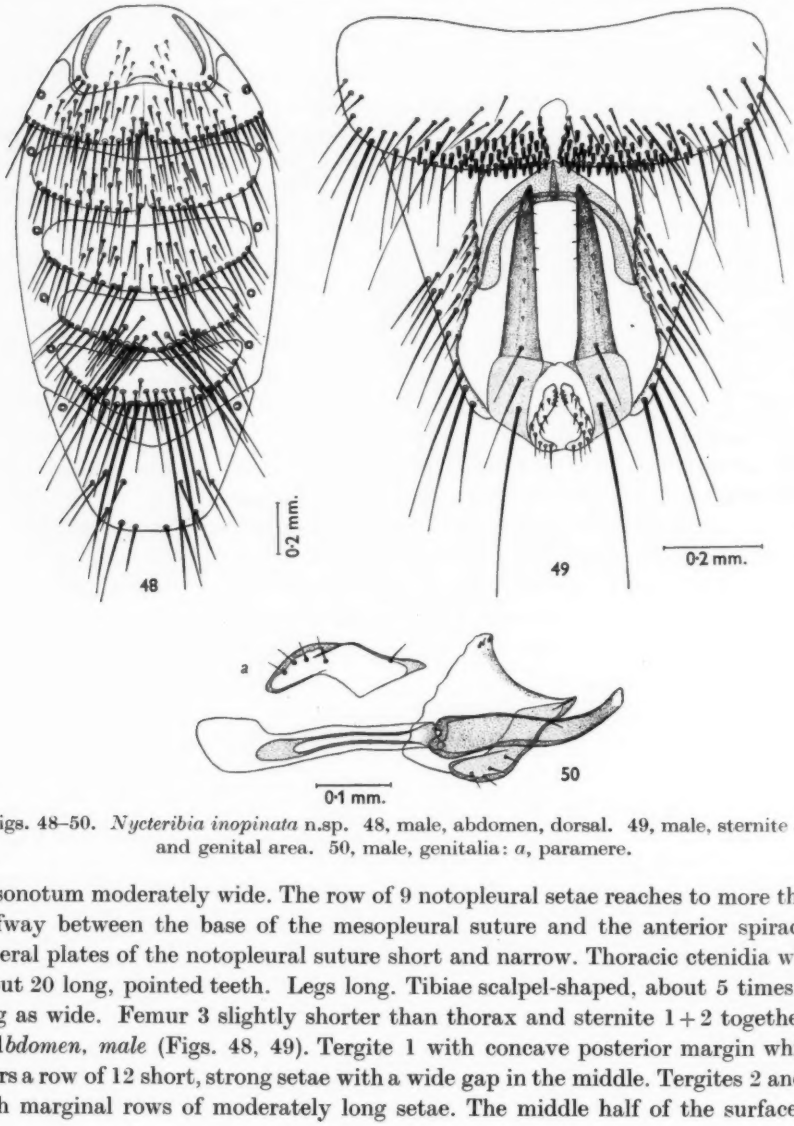
Subspecies (?). *S. Rhodesia*: Odzi, *Hipposideros caffer*, 1 ♀ (Miller), *Rhinolophus darlingi*, 1 ♂ (Miller). *Transvaal*: Onderstepoort, Pretoria, *R. darlingi*, 1 ♀ (Bedford).

12. **Nycteribia inopinata** n.sp.

Length 2·5 mm. Colour brown.

Head not compressed laterally, sclerotized up to the anterior dorsal margin which bears a row of about 10 setae. Labella of labium about as long as the theca.

Thorax as long as wide. Sternal median suture well marked, widened in middle. Angle of oblique sutures about 80°. 3 setae at each side of the posterior margin of the sternal plate. The middle seta of each group is about as long as sternite 1+2.



Figs. 48–50. *Nycteribia inopinata* n.sp. 48, male, abdomen, dorsal. 49, male, sternite 5 and genital area. 50, male, genitalia: *a*, paramere.

Mesonotum moderately wide. The row of 9 notopleural setae reaches to more than halfway between the base of the mesopleural suture and the anterior spiracle. Lateral plates of the notopleural suture short and narrow. Thoracic ctenidia with about 20 long, pointed teeth. Legs long. Tibiae scalpel-shaped, about 5 times as long as wide. Femur 3 slightly shorter than thorax and sternite 1+2 together.

Abdomen, male (Figs. 48, 49). Tergite 1 with concave posterior margin which bears a row of 12 short, strong setae with a wide gap in the middle. Tergites 2 and 3 with marginal rows of moderately long setae. The middle half of the surface is

covered with short setae. Tergites 2 and 3 are incompletely divided in the middle, tergites 4–6 undivided. There are 4 long setae in the middle of the marginal rows of tergites 5 and 6. Tergite 4 is covered with short setae in the middle like tergites 2 and 3. Tergites 5 and 6 are bare on the surface except for a premarginal row of short setae. Anal segment wide and rounded with deeply concave anterior dorsal margin. A double transverse row of short setae in the middle of the dorsal surface with a gap in the middle. Sternite 1 + 2 with a ctenidium of 47 moderately long teeth. Posterior part of the surface with 2–3 rows of short setae in the middle and 4–5 rows laterally. Sternites 3 and 4 with marginal rows of long and short setae and short setae on the surface. Sternite 5 longer than sternite 4, with a deep median indentation of the posterior margin which widens anteriorly. 2 groups of short spines near the indentation, each consisting of about 4–5 rows. All spines of about equal length.

Genitalia (Fig. 50). Claspers tapering uniformly, pigmented with darker tips. 3 long setae dorsally near the base. Basal arc wide, rounded with a short anterior process. Basal plate wide and short, slightly curved. Aedeagus wide in dorsal view, curved in profile. Apodeme short with moderately wide end-plate. Parameres with short apical process and a single short seta at the base of the process at the ventral edge. A row of 4 short setae in the basal part.

Female unknown.

Material examined

1 ♂ (type), *Rhinolophus alcyone*, Nkoétyé near Ebolowa, Cameroons. (Perret.) Muséum d'Histoire Naturelle, Geneva.

N. inopinata resembles *N. scissa* in habitus and particularly in the form of sternite 5. It belongs, however, to the *biarticulata* group according to the row of setae at the posterior margin of the thorax and the row of notopleural setae. It differs from *N. tecta* mainly in the greater number of setae on the abdominal tergites and in the absence of long spines in the posterior row of spines on sternite 5.

13. **Nycteribia rotundata** n.sp.

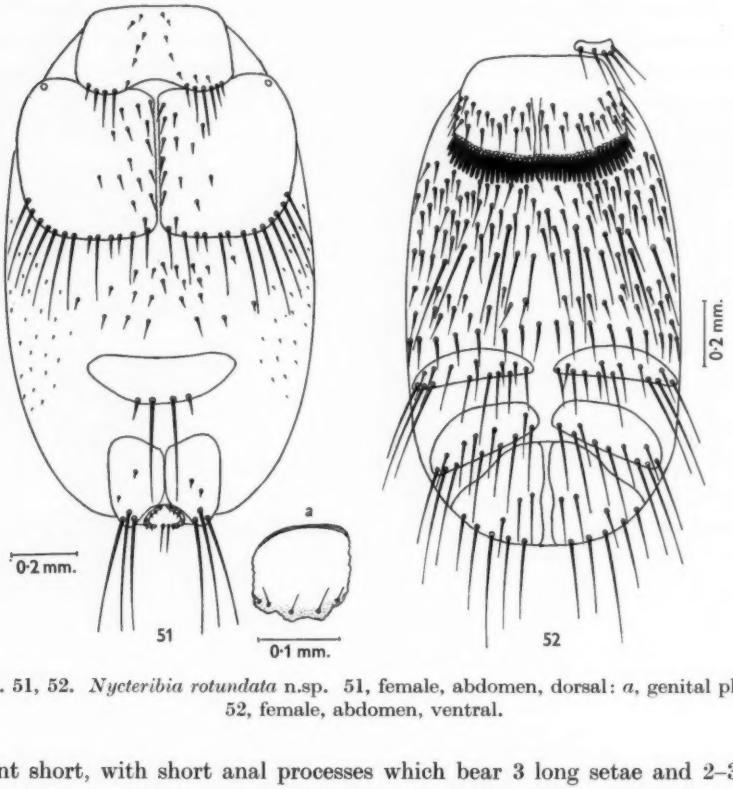
Length 2·5 mm.

Head. 4 setae at the anterior dorsal margin. Labella of the labium markedly longer than the theca.

Thorax as long as wide. Median sternal suture widened in the middle. Angle of oblique sutures about 80°. 4 setae at each side of the posterior margin of the sternal plate of the thorax, of which one is very long. A row of 8 notopleural setae which reaches to halfway between the base of the mesopleural suture and the anterior spiracle. The anterior setae are much more widely spaced than the posterior ones. Legs even shorter than in *N. tecta*. Femur 3 shorter than thorax and sternite 1 + 2 together.

Abdomen, female (Figs. 51, 52). Tergite 1 concave posteriorly with 4–5 short setae in each half and a wide gap between them. Tergal plate 2 twice as long as tergite 1, divided in middle, each half rounded, with a marginal row of about 10

setae in each half which are shorter and more widely spaced towards the middle and longer and thinner laterally. Some short spines between the setae near the middle. About 3 rows of short spines on the surface near the median line. Tergal plate 3 wide, in the shape of a narrow circle segment with 2 long (?) setae and 2 short spines at the posterior margin (setae missing). Only 3–4 rows of short spines between tergal plates 2 and 3. Pleurae with widely spaced minute spines. Anal



Figs. 51, 52. *Nycteribia rotundata* n.sp. 51, female, abdomen, dorsal: *a*, genital plate. 52, female, abdomen, ventral.

segment short, with short anal processes which bear 3 long setae and 2–3 very short spines near the base on the dorsal surface. Sternite 1 + 2 much narrower than the abdomen with a ctenidium of 44 short teeth and 2–3 rows of short spines on the surface. Several spines of the posterior row of the surface longer. Post-spiracular sclerite large, parallel-sided, with 5 setae at the ventral margin. Sternite 3 with a marginal row of moderately long setae and 1–2 longer setae at each side. 4–6 rows of short setae on the surface and 4 long setae in the posterior row of the surface. Sternite 4 with shorter marginal setae and 1–2 rows of short setae on the surface. Sternite 5 divided into 2 narrow lateral sclerites which reach nearly to the median line. They bear a row of long and short setae posteriorly and a row of short setae on the surface. No setae between the sclerites. Sternite 6 with similar but broader sclerites. Sternite 7 wide, triangular, with a row of long setae posteriorly and a curved row of setae across the middle. Dorsal genital plate rectangular,

with 5 minute hairs posteriorly and more heavily sclerotized along the anterior and posterior margin. Anal sclerite absent, replaced by two setae.

Male unknown.

Material examined

Rhodesia: Mazoe, *Rhinolophus hildebrandti*, 1 ♀ (Darling) (type), British Museum (Nat. Hist.)

The species resembles *N. integra* in the shape of the genital plate.

14. **Nycteribia ovalis** n.sp.

Length 2 mm.

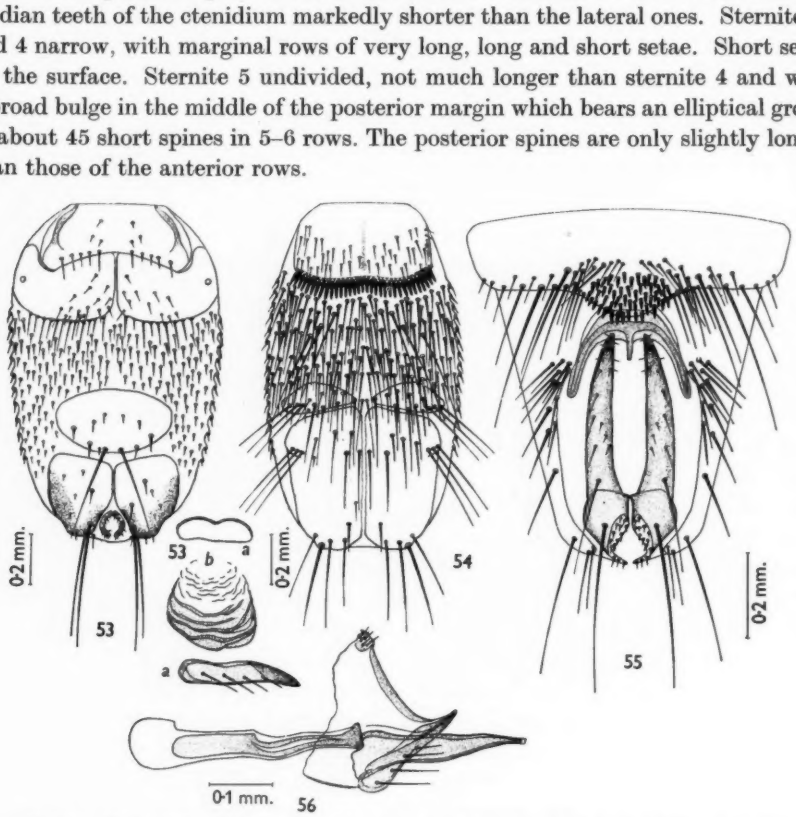
Head. 6–8 setae at the anterior dorsal margin. Labella of labium as long as the theca.

Thorax slightly longer than wide. Median sternal suture not widened in middle. Angle of oblique sutures about 75°. Mesonotum narrow. A row of 8–10 notopleural setae which reach to halfway between the base of the mesopleural suture and the anterior spiracle. 3 setae at each side of the posterior margin of the sternal plate, the middle one of each group longer. Legs short, as in *N. rotundata*.

Abdomen, female (Figs. 53, 54). Tergite 1 with slightly concave posterior margin which bears a row of about 10 short setae with a gap in the middle. Tergal plate 2 short, divided in the middle. The posterior margin bears a row of about 10 short setae and some short spines in each half. Tergal plate 3 very large, elliptical, with 2 long setae in the middle of the posterior margin and 2–3 short spines at the sides. A row of 4 short spines in the middle of the surface. Dorsum of the abdomen between tergal plates 2 and 3 and pleurae covered with rather long spines. Anal segment large, rounded, divided into 2 sclerites which bear 2 long setae posteriorly and several small spines on the dorsal surface. Sternite 1 + 2 narrow, rectangular, with a ctenidium of 36 rather short teeth. Postspiracular sclerite narrow, curved, with about 5 long setae. Sternites 3 and 4 with marginal rows of moderately long setae. 2 setae at the sides are very long. Surface densely covered with setae which are not much shorter than those of the marginal rows. 4 long setae in a transverse row among the shorter ones on the surface of sternite 3. Sternite 5 with 2 narrow elliptical sclerites with a row of long and short setae posteriorly and shorter setae on the surface. 2 setae between the sclerites. Sternites 6 and 7 divided into lateral sclerites. The sclerites of each side of both sternites fused into an oblong plate. The arrangement of setae on the part of the plate corresponding to sternite 6 as on sternite 5. The sclerites of sternite 7 are bare except for 4 long setae posteriorly and a few minute spines along the median division line. Dorsal genital plate unarmed, rectangular, forming a lip which covers the genital opening from above. Ventral plate large, concave on the posterior surface and with transverse ridges and scales.

Abdomen, male (Fig. 55). Tergite 2 incompletely divided in the middle with a marginal row of moderately long setae and short setae in the median half of the surface. Tergites 3–6 with widely spaced short setae at the posterior margin. Only 2 setae in the middle of the row on tergite 5 are longer. About 6 short setae in each

half near the median line on tergite 3, 2–3 on tergite 4, tergites 5 and 6 bare. Anal segment conical, with 3–4 long setae posteriorly and a transverse row of 4 short setae in the posterior part of the dorsal surface. Sternite 1 + 2 as in the female, median teeth of the ctenidium markedly shorter than the lateral ones. Sternites 3 and 4 narrow, with marginal rows of very long, long and short setae. Short setae on the surface. Sternite 5 undivided, not much longer than sternite 4 and with a broad bulge in the middle of the posterior margin which bears an elliptical group of about 45 short spines in 5–6 rows. The posterior spines are only slightly longer than those of the anterior rows.



Figs. 53–56. *Nycteribia ovalis* n.sp. 53, female, abdomen, dorsal: *a*, dorsal; *b*, ventral genital plates. 54, female, abdomen, ventral. 55, male, sternite 5 and genital area. 56, male, genitalia: *a*, paramere, dorsal.

Genitalia (Fig. 56). Basal arc large, rounded, with a short posterior process. Claspers slightly curved, with a long seta dorsally near the base and 2–3 shorter setae further anteriorly. Some short setae near the tip. Phallobase conical, with 2 groups of short setae near the base. Aedeagus straight, tapering to a blunt point. Apodeme with a narrow end-plate. Parameres long, partly fused with the phallobase, with long, pointed, pigmented distal process and 4–5 rather long setae in the basal part.

Material examined

Sierre Leona: Makeni, *Hipposideros caffer*, 1 ♂, 1 ♀ (types) (Jones). *Natal*: Pietermaritzburg, *Rhinolophus clivosus augur* or *Nycteris capensis*, 1 ♂ (Lawrence). *Tanganyika*: Manda, *Rhinolophus* sp., 1 ♀.

Genus **Basilia** Mir. Ribeiro, 1903

Subgenus **Basilia** s.str.

Head laterally compressed, of *Nycteribia* type. Eyes consisting of 2 ocelli with common pigmented base. *Thorax* of *Nycteribia* type. Legs moderately long, tibiae scalpel-shaped, with long setae in the distal part of the ventral edge which reach beyond the tip of the tibia. Tergal plate 2 of female abdomen large, either with posterior processes with long setae or with straight posterior margin. Genital plate reduced to a group of setae on a field of minute hairs. Posterior tergites of the abdomen of the male generally with some very long setae. Sternite 5 with an armature of spines at the posterior margin. Genitalia of *Nycteribia* type.

All species of this subgenus known so far from the Ethiopian Region belong to the *bathybothyra* group which has been dealt with in detail in another paper (Theodor, 1956).

Key to the species of the subgenus Basilia s.str.

Females

1. Posterior setae of tergite 1 of the abdomen very short and thick, less than one-third of the length of the long setae of tergal plate 2. Anal sclerite with 8–10 setae (Fig. 65). **Basilia meridionalis** Theodor
— Setae of tergite 1 half as long as those of tergal plate 2 or longer. **2**
2. Small species (2 mm.). Setae of tergite 1 nearly as long as those of tergal plate 2. Tergal plate 3 with long posterior processes which bear a long seta and 2 short spines. Anal segment longer than wide (Fig. 57). **Basilia ansifera** Theodor
— Larger species (2·5 mm.). **3**
3. Setae of tergite 1 nearly as long as those of tergal plate 2. Anal segment short, about as long as wide. Anal sclerite reduced to 1 to 2 isolated setae. Middle East, Kenya (?). **Basilia daganiae** Theodor & Moscona
— Setae of tergite 1 markedly shorter than those of tergal plate 2 (one-half or two-thirds). Anal segment longer than wide. Anal sclerite well developed, with 2 longer and some shorter setae (Fig. 61). **Basilia robusta** Theodor

Males

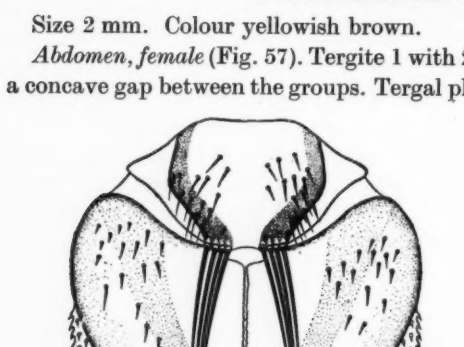
1. Small species (2 mm.). Tergite 2 of abdomen triangularly produced posteriorly, with a group of 8–10 closely standing long setae in the middle. Parameres with long curved distal end (Figs. 58–60). **Basilia ansifera** Theodor
— Larger species (2·5 mm.). Tergite 2 uniformly rounded posteriorly, with a row of setae of about equal length. **2**
2. Spines of abdominal ctenidium very slender, 10 times as long as wide. No gap in marginal row of tergite 6. **Basilia tenuispina** n.sp.
— Spines wider, 7 times as long as wide. A gap in the marginal row of tergite 6. **3**
3. Strongly sclerotized. Abdomen wide, tergites 4–6 short. Anal segment longer than the 3 preceding segments. Some small spines in the middle of the surface of tergite 2. The group of spines on sternite 5 consists of 22–27 spines. Aedeagus straight, tapering to a truncated point. Parameres with a blunt, truncate distal end (Figs. 62–64). **Basilia robusta** Theodor
— Weakly sclerotized. Abdomen narrower, tergites 4–6 longer. Anal segment short, as long as the 2 preceding segments. Middle of surface of tergite 2 bare. Group of spines on sternite 5 consisting of 13–16 spines. Aedeagus curved, tapering to a fine point. Parameres with sharp distal point and small ventral tooth. **Basilia daganiae** Theodor & Moscona

The male of *Basilia meridionalis* is unknown.

1. **Basilia ansifera** Theodor, 1956

Size 2 mm. Colour yellowish brown.

Abdomen, female (Fig. 57). Tergite 1 with 2 groups of 3–5 very long setae and with a concave gap between the groups. Tergal plate 2 heart-shaped, with long posterior



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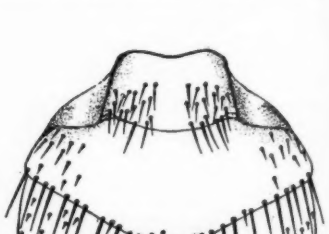

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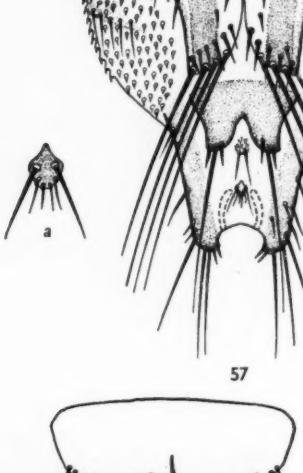

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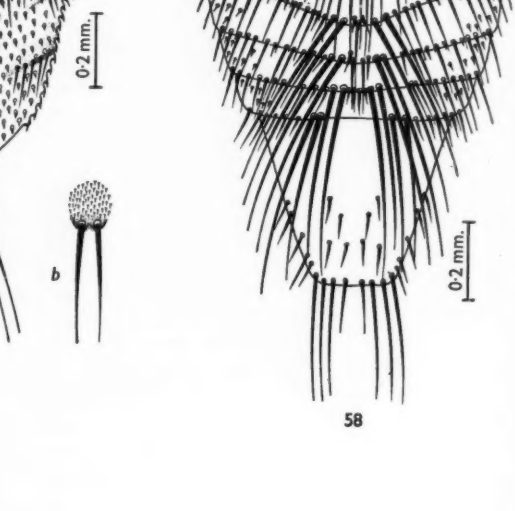

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Figs. 57–60. *Basilia ansifera*. 57, female, abdomen, dorsal: *a*, anal sclerite; *b*, genital plate. 58, male, abdomen, dorsal. 59, male, *a*, sternite 5 and genital area; *b*, anal frame. 60, male, genitalia.

processes which bear 3–5 very long and thick setae. They are only slightly longer but much thicker than those of tergite 1. Short spines on the surface of the plate. Tergal plate 3 with rather long posterior processes which bear a long seta and 2 short spines. Anal segment longer than wide, nearly parallel-sided. Pleurae covered with minute spines. Abdominal ctenidium with 60–70 long teeth. Sternites 5 and 6 with 2 lateral sclerites. Anal sclerite drop-shaped with 2 longer setae at the widest part and 4–5 short setae posteriorly. Genital plate small, rounded, with minute hairs and 2–4 setae posteriorly.

Abdomen, male (Figs. 58, 59). Tergite 2 triangularly produced posteriorly with a group of 8–10 closely standing long setae in the middle. 2–4 very long setae in the middle of the marginal rows of tergites 3–6. Surface of tergites 3–6 bare except for some minute spines at the sides of the surface. Sternite 5 with a double row of 16–24 short spines in the middle of the posterior margin.

Genitalia (Fig. 60). Aedeagus straight and of about equal width in the basal two-thirds and curved and tapering to a point in the apical third. Parameres with a long, slightly curved distal process, a blunt ventral tooth and a shallow concavity between them.

Distribution. Mainly West Africa, Belgian Congo, eastwards to Lake Victoria, Lake Nyanza and to Torit, Equatoria province, Sudan, nearly all between 10° lat. north and south (Map no. 3, p. 542).

Hosts. Pipistrellus culex, P. helios, P. minusculus, P. nanus, P. stampflii, Pipistrellus sp., *Eptesicus rendalli, E. tenuipinnis, Scotophilus* sp., *Tadarida pumila.*

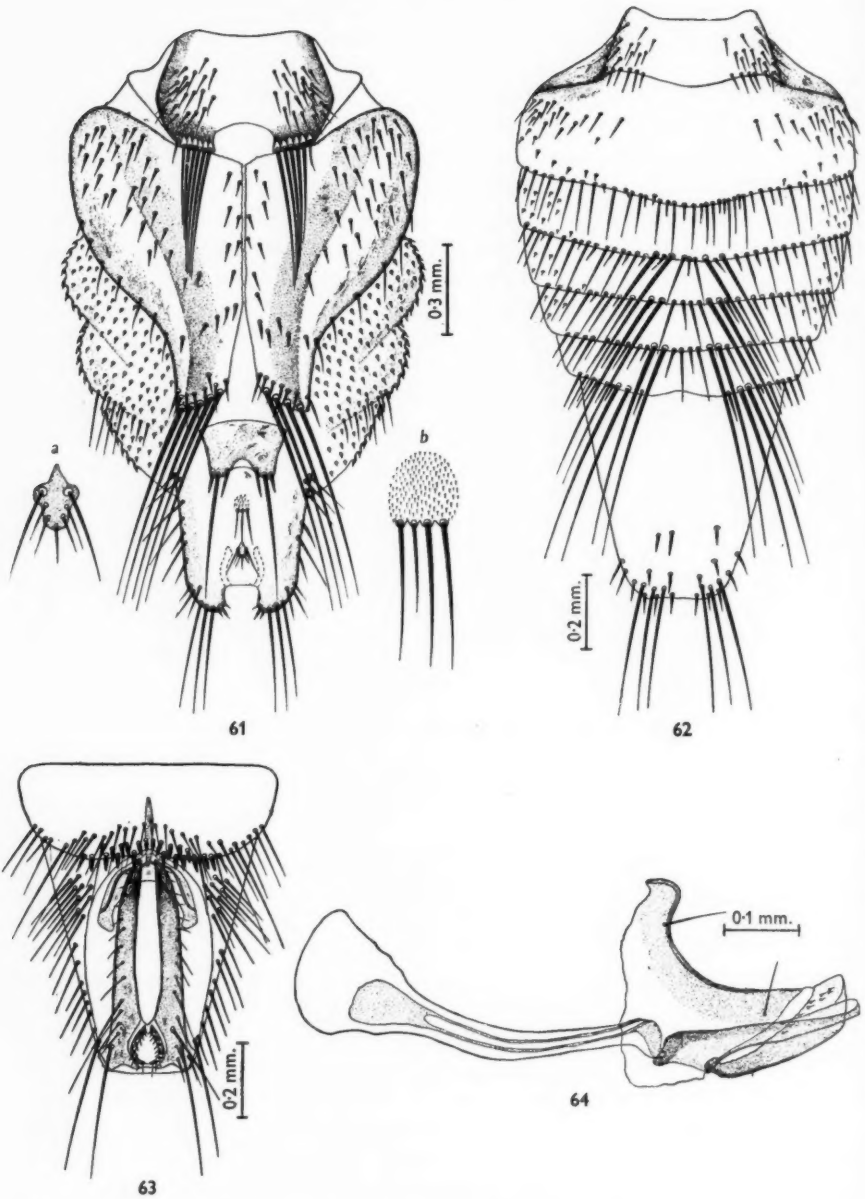
Full details of the localities of the material examined have been given with the original description.

2. **Basilia robusta** Theodor, 1956

Length 2·2–2·5 mm. Colour brown, heavily sclerotized. Labella of labium very short, about one-third of the length of the theca. Thorax markedly wider than long. 10–12 notopleural setae.

Abdomen, female (Fig. 61). Tergite 1 with 2 groups of 4–5, rarely 6–7 moderately long setae. Tergal plate 2 with wide truncate posterior processes which bear 4–5 very long and thick setae. The setae of tergite 1 are half as long as those of tergal plate 2 in the Abyssinian specimens and two-thirds of their length in the specimens from Uganda and the Congo. Tergal plate 3 with 2 broad processes which bear 1–2 long setae and 1 or 2 short setae or spines. Anal segment longer than wide, bare dorsally and with rather long anal processes. Sternite 1 + 2 with a ctenidium of 65–70 long teeth. Anal sclerite small and of irregular form, wider in the middle, with 2 longer setae and 4 shorter setae posteriorly. Genital plate with about 4 long setae.

Abdomen, male (Figs. 62, 63). Tergite 2 uniformly rounded posteriorly, without the bulge in the middle present in *B. ansifera*. The marginal row consists of uniformly spaced setae of about equal length. The median setae are not markedly longer than the lateral ones as in *B. ansifera*. Tergites 3–6 very narrow with 4 very long setae in the middle of the marginal rows. Anal segment as long as the 3 or 4 preceding tergites. Sternite 5 with a double row of 22–27 short spines in the middle of the posterior margin.



Figs. 61–64. *Basilia robusta*. 61, female, abdomen, dorsal: *a*, anal sclerite; *b*, genital plate. 62, male, abdomen dorsal. 63, male, sternite 5 and genital area. 64, male, genitalia.

Genitalia (Fig. 64). Aedeagus nearly straight, tapering to a truncate tip. Parameres with truncate distal end.

Material examined

Abyssinia: Harar, *Pipistrellus nanus*, 2 ♂, 4 ♀ (Kristensen). *Uganda*: Bubembe Island, Lake Victoria, 8 ♀, 3 ♂ (Pitman). *Belgian Congo*: Bitshumbi, Lake Edward, 1 ♀ (de Witte); Uvira, Kivu, Lukuka cave, 2 spec. (Leleup). *S. Rhodesia*: Odzi district, *Pipistrellus kuhlii*, 1 ♂ (Miller). *Angola*: Huila, 1 ♂, 1 ♀, (Heinrith).

3. **Basilia daganiae** Theodor & Moscona, 1954

Syn. *Basilia bathybothyra daganiae* Theodor & Moscona, 1954

This species was originally described from Palestine and occurs also in Cyprus and Egypt on *Pipistrellus kuhlii*. It resembles *Basilia robusta* in some respects. It differs from it in the female in its shorter anal segment, which is not longer than wide, in the reduction of the anal sclerite to 1 or 2 isolated setae and in the greater length of the setae of tergite 1, which are nearly as long as those of tergal plate 2. In the male the abdominal tergites 3–6 are longer than in *B. robusta*. The row of spines on sternite 5 consists of only 13–16 spines. The aedeagus is curved and resembles that of *B. ansifera* and the parameres have a short, sharp, distal point.

A single male which may belong to this species was found on *Cardioderma cor* in Mombasa, Kenya. This specimen differs in some details from the Middle East form, mainly in having only a single row of 9 spines at the posterior margin of sternite 5. More material is necessary to determine the status of this form.

4. **Basilia meridionalis** Theodor, 1956

Length 2·5 mm. Colour brown, strongly sclerotized.

Abdomen, female (Fig. 65). The posterior setae of tergite 1 are very short and thick, about one-third of the length of the long setae of tergal plate 2. Tergal plate 3 with short and broad posterior processes which bear a long seta and 2 short spines. Anal segment longer than wide, bare dorsally and laterally except for 1 or 2 short spines near the base of the anal processes. Anal sclerite broad, with 2 longer setae and 7–8 short setae posteriorly. Genital plate transversely elliptical with 2 long and 3 shorter setae.

Male unknown.

Material examined

A single female from South Africa without exact locality and host.

5. **Basilia tenuispina** n.sp.

Length 2·7 mm. Colour yellowish brown.

Head long, laterally compressed, sclerotized up to the anterior margin which bears 6 setae. Eyes with incompletely separated ocelli. A row of short setae along the anterior ventral margin which become gradually longer posteriorly, ending at the base of the palps. A single seta further back. Palps with very long terminal seta. Labella of labium half the length of the theca.

Thorax. Length to width = 5:6. Median ventral suture weakly developed, not

widened in the middle. Oblique sutures forming an angle of about 90°, narrowly open, ending some distance before the margin. The row of setae at the posterior ventral margin consists of 4–5 very long setae at each side and shorter setae between them. Mesonotum moderately wide. Notopleural sutures thin. 8 notopleural setae of moderate length. Legs: Tibiae scalpel-shaped with 3 rows of setae near the distal end, 4 times as long as wide. The setae of the distal row are long and strong and reach beyond the tip of the tibia (Fig. 66).

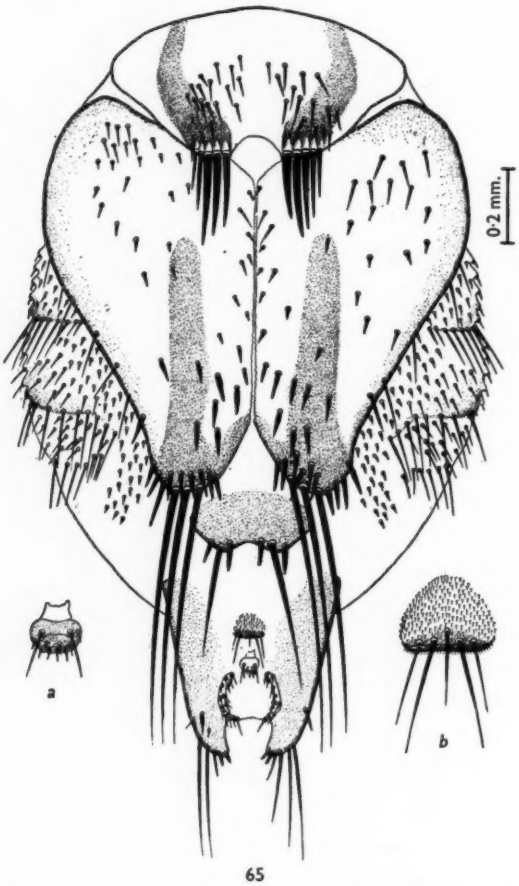


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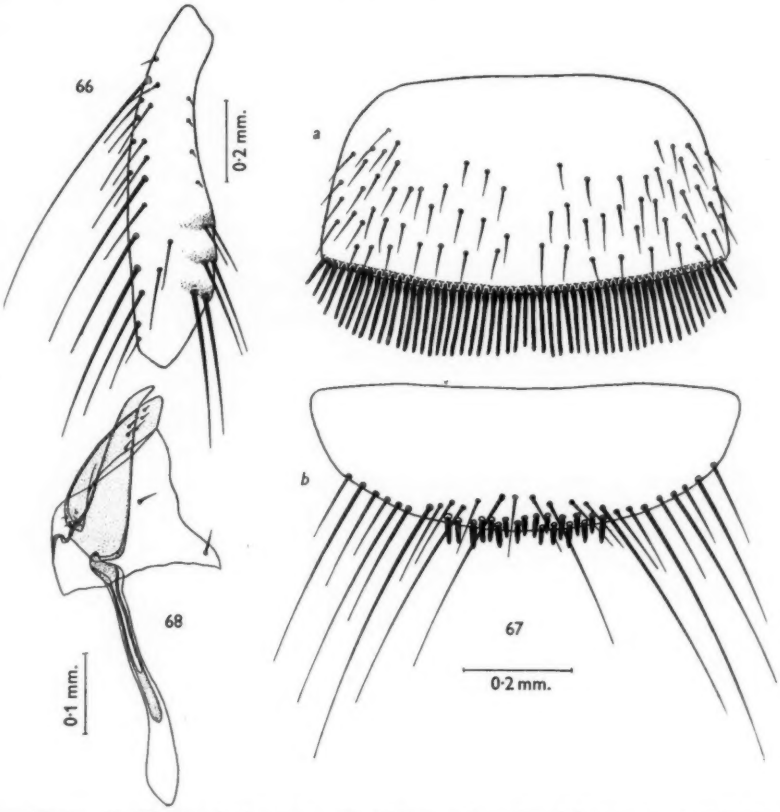
Fig. 65. *Basilia meridionalis*, female, abdomen, dorsal: *a*, anal sclerite; *b*, genital plate.

Abdomen, male (Fig. 67). Tergite 1 with straight posterior margin which bears a row of about 8 closely standing setae near its lateral corner. There is a wide gap between the two rows. Tergite 2 broadly rounded with a marginal row of moderately long, thin setae and a few spines. An irregular double row of short spines runs across the middle of the surface of the tergite and there are a few short spines in the middle behind this row. Tergite 3 with a similar marginal row, bare on the surface,

except for a few short setae at the sides of the surface. Tergites 4–6 bare on the surface and with similar marginal rows, but with 4–6 very long setae near the middle of the rows. There is no gap in the middle of the marginal row of tergite 6. Anal segment conical with some long and shorter setae in the posterior part of the dorsal surface and some long setae at the posterior margin. Sternite 1 + 2 rectangular, with a ctenidium of about 50 very slender teeth. These teeth are about 10 times as long as wide. 3–4 rows of short setae in the posterior part of the



Figs. 66–68. *Basilia tenuispina* n.sp. 66, tibia 3. 67, male, abdomen: *a*, sternite 1 + 2; *b*, sternite 5. 68, male, genitalia.

surface. Postspiracular sclerite with 3 long setae towards the spiracle. Sternite 3 with a marginal row which consists of 3 very long setae at each lateral corner and shorter setae towards the middle. 2 long setae stand close to the marginal row in the middle of the surface. The surface is covered with very short setae in the middle and longer setae laterally. Sternite 4 with a similar marginal row, but the surface is quite bare except for 4 long setae close to the marginal row. Sternite 5 longer than sternite 4, with a rounded posterior margin. There is a double row of 18 rather long, thick spines in the middle of the posterior margin, 10 longer spines in

the posterior row and 8 shorter spines in the anterior row. There is a marginal row of very long and shorter setae at the sides of the posterior margin which continues on the surface anterior to the rows of spines.

Genitalia (Fig. 68). Claspers slightly curved, tapering to a pigmented tip. A row of moderately long setae near the base on the dorsal surface. Basal arc weakly developed, rounded, with a short anterior process. Phallobase short, conical, with 2 short setae near the base and 2 short setae at the middle. Aedeagus thick at the base, tapering to a sharp curved tip. Apodeme short with a narrow end-plate. Parameres slender, slightly curved, with rounded tip and a double row of minute hairs in the distal half.

Female unknown.

Material examined

Belgian Congo: Nia Nia, near Stanleyville, *Epomops franqueti*, 1 ♂, type.

This species will possibly prove to belong to the subgenus *Paracyclopodia*, the species of which have a continuous marginal row on tergite 6 of the male. This cannot, however, be decided as long as the female is unknown.

Subgenus **Paracyclopodia** Scott, 1917

Basilia (*Paracyclopodia*) *roylii*, the type species of the subgenus, was placed by Scott (1908) in the genus *Cyclopodia* because of its relatively long tibiae with short setae and the presence of eyes with 2 ocelli. Scott (1917) recognized the marked differences from *Cyclopodia* and created for *Paracyclopodia roylii* the subgenus *Paracyclopodia* of the genus *Cyclopodia*. Theodor (1954) removed the subgenus from *Cyclopodia* and considered it provisionally as a separate genus, as the structure of head and eyes, thorax and male genitalia clearly proved that this species did not belong to the genus *Cyclopodia* and was much more closely related to *Basilia*. *B. roylii* differs from other species of *Basilia* mainly in the peculiar segmentation of the abdomen of the female in which tergal plate 1 and sternite 1+2 are very long, covering nearly two-thirds of the abdomen, and the other segments and spiracles 2–7 are concentrated in the posterior third of the abdomen. *Basilia bouvieri*, redescribed in this paper, is in some respects transitional between *Paracyclopodia roylii* and other species of the genus *Basilia*. The structure of head and eyes, the structure of thorax and the male genitalia are very similar to those of the genus *Basilia* and the segmentation of the female abdomen is intermediate between that of *Paracyclopodia roylii* and other species of *Basilia*. It is very difficult to distinguish males of the subgenus *Paracyclopodia* from those of the subgenus *Basilia*. The differences in the legs are not very marked. The differences in the segmentation of the female abdomen alone, however, do not seem to justify the retention of *Paracyclopodia* as a separate genus. The peculiar segmentation of the female abdomen in *P. roylii* seems to be of specific rank and the existence of a transitional form, together with the close similarity of the males and the females to the genus *Basilia* in the characters mentioned above, therefore justify the inclusion of *Paracyclopodia* as a subgenus in the genus *Basilia*.

Diagnosis. Head, eyes, thorax, abdomen of the male and male genitalia, as in

the subgenus *Basilia*. Tibiae tapering gradually towards the tip, with short setae in the middle (Fig. 69). The abdomen of the female has a long first tergal plate and sternite 1+2. All other segments are concentrated in the posterior half or third of the abdomen and are strongly modified in shape. Sternite 7 stands vertical to the ventral surface and functions as genital plate.

6. **Basilia (Paracyclopodia) bouvieri** Falcoz, 1924

Syn. *Tripselia* (*Neotripselia*) *bouvieri* Falcoz, 1924

This species was described by Falcoz (1924) from 2 males from West Africa without exact locality or host. Falcoz created for them the new subgenus *Neotripselia* as they appeared to have eyes consisting of a single ocellus. Re-examination of the type proved, however, that the eyes consist of 2 not very clearly separated ocelli. The species belongs to the subgenus *Paracyclopodia* of the genus *Basilia* according to the structure of the female abdomen.

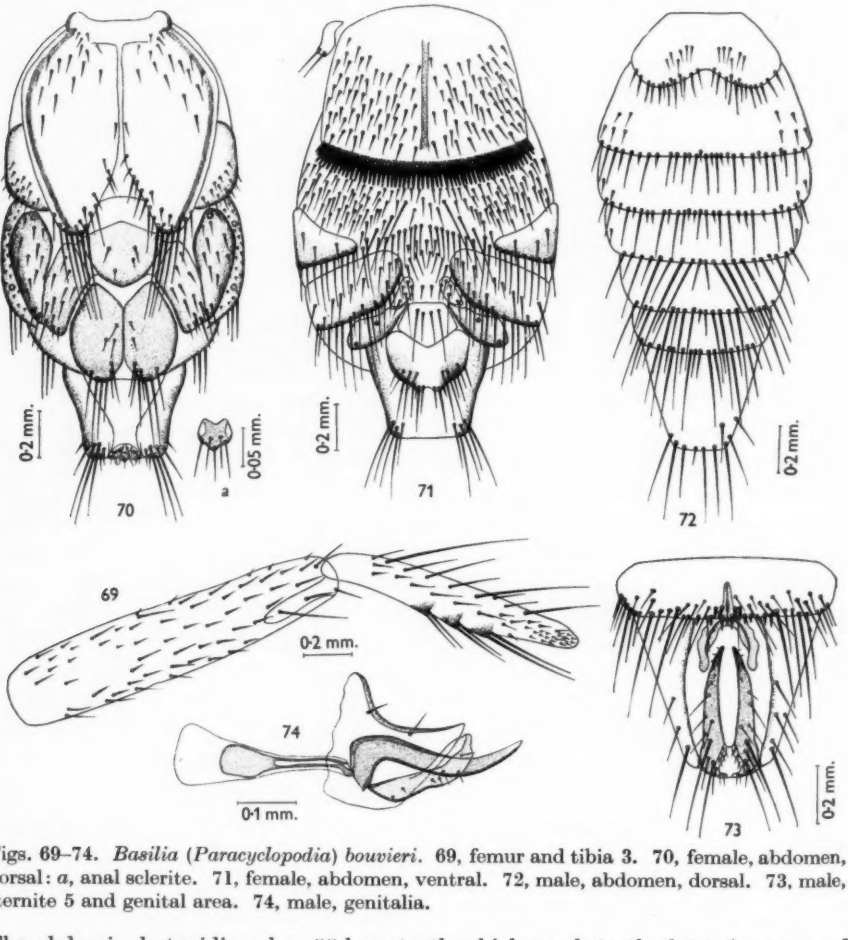
Length 2·2–2·7 mm. Colour brown.

Head long, laterally compressed, sclerotized up to the anterior margin with eyes of *Basilia* type, i.e. 2 ocelli on a common pigmented base, which are not clearly separated. About 6 setae at the anterior dorsal margin and 2 further posteriorly between the eyes. Short setae at the anterior ventral margin and a longer seta posteriorly. Only 1 or 2 short setae on the genae. Palpi long and slender with a relatively short terminal seta, 3 shorter setae near the tip and 3–4 still shorter setae along the stem. Labella of the labium slightly shorter than the theca.

Thorax slightly wider than long. Sternal plate nearly square. Median ventral suture narrow, not widened in the middle. Angle of oblique sutures about 95°. They are widely open and end some distance from the margin. Posterior margin of the sternal plate with short setae and 2 longer ones at the sides. Mesonotum moderately wide. 9–12 rather short notopleural setae. Legs (Fig. 69): tibiae slender, 5 times as long as wide, gradually tapering from the middle, with 3 rows of setae in the middle of the ventral surface. The distal row consists of long setae which just reach the tip of the tibia, the middle row is shorter and the basal row still shorter. Femur 1 is scarcely longer than the tibia, femurs 2 and 3 longer by about one-third than the tibiae.

Abdomen, female (Figs. 70, 71). Tergite 1 long, with curved sides, reaching to about the middle of the abdomen, divided by a median line, with two rounded posterior processes which bear 4–5 long and several shorter setae. These processes are widely separated and do not touch each other as in the species of *Basilia* described. Short setae along the inner posterior margin of the tergal plate. A pigmented line runs along the outer margin of the plate. Short setae in the anterior part of the surface and near the posterior inner margin. Tergal plate 2 is W-shaped. The outer arms of the W are triangular plates which are wider in front. Their surface is directed laterally and is covered with short setae and some longer setae in the posterior angle. Spiracle 2 is situated at the anterior margin of this plate, spiracle 3 a short distance laterally in the pleural membrane. There is no sclerite around it as in *B. roylii*. The middle part of the W is formed by a broad plate which is covered with short spines near its junction with the lateral parts of

the plate. 2 pairs of short setae in the middle. Tergal plate 3 is wedged into the posterior concavity of tergal plate 2 or even partly fused with it. It consists of 2 triangular plates which bear several long and short setae posteriorly and a few short setae in the middle near the median line. Anal segment short, conical, with long and short setae posteriorly, otherwise quite bare. Anal sclerite short and broad, triangular, with 3 setae posteriorly. Some minute, widely spaced spines on the pleurae. Sternite 1 + 2 rectangular, long, covering less than half the abdomen.



Figs. 69–74. *Basilia* (*Paracyclopodia*) *bouvieri*. 69, femur and tibia 3. 70, female, abdomen, dorsal: *a*, anal sclerite. 71, female, abdomen, ventral. 72, male, abdomen, dorsal. 73, male, sternite 5 and genital area. 74, male, genitalia.

The abdominal ctenidium has 55 long teeth which reach to the lateral corners of the sclerite. About 5 rows of short spines on the surface. Postspiracular sclerite widening towards the spiracle, with 2 long setae. Sternite 3 membranous, with concave posterior margin and with 2 triangular sclerites in the lateral posterior corners. These plates bear a row of moderately long setae posteriorly which continue between them in a dense row. A few short setae on the surface of the sclerites and 3–4 rows of short setae on the surface of the membranous part of the sternite.

7 longer setae in the posterior row of the surface near the middle and 2 more further anteriorly. Sternite 4 similar, but the sclerites are larger, elliptical, stand obliquely and reach closer to the median line. The setae at the posterior margin are longer and there are only 4–6 setae between the sclerites and a few short setae anterior to them. 2 rows of short setae on the surface of the sclerites. Sternite 5 reduced to 2 small elliptical sclerites near the inner corners of the sclerites of sternite 4. They bear 4–5 short setae and a longer one on the surface. 2 short setae between the sclerites in some specimens. Sternite 6 consists of 2 obliquely standing, elliptical sclerites which are quite bare and a pentagonal sclerite between them. Spiracles 6 and 7 lie inside these lateral plates. A group of 4–5 setae on the median sclerite of which the 2 lateral setae are longer. Sternite 7 rounded posteriorly, standing vertically to the ventral surface, with longer and shorter setae posteriorly and 2 long and several shorter setae in the posterior lateral corners of the surface. This sclerite functions as genital plate, covering the genital opening from below. A genital plate seems to be absent, but there is a field of minute spines as in other species of *Basilia* without, however, any setae.

Abdomen, male (Figs. 72, 73). Tergite 1 with 2 rounded posterior lobes and a marginal row of short setae with a small gap in the middle. Some long spines on the surface. Tergite 2 with straight posterior margin which bears a row of short setae and some short spines. Only 6–8 short spines in the lateral parts of the surface. Tergite 3 similar but the marginal setae are longer. Tergites 4–6 with marginal rows of long setae, shorter setae and short spines. There is a small or no gap in the middle of the marginal row of tergite 6. The long setae are about as long as 2 tergites. Surface of the tergites bare. Anal segment short, conical, bare on the dorsal surface and with some long setae posteriorly. Sternite 1+2 as in the female but shorter, with a ctenidium of about 45–50 teeth. Sternites 3 and 4 narrow, with marginal rows of short setae on sternite 3 and longer and shorter setae on sternite 4. Several rows of short spines on the surface of the sternites. Sternite 5 short, rectangular, not longer than sternite 4, with long setae at the straight posterior margin and a double row of short spines. There are about 8 spines in the posterior row and 5–6 more closely standing spines in the anterior row. The two median spines of the posterior row are larger than the others.

Genitalia (Fig. 74). Claspers rather short, slightly curved, with a few long setae dorsally near the base. Basal arc rounded with long anterior process. Phallobase short, conical with 2 shorter setae near the base and 2 longer setae in the middle. Aedeagus with sharp basal curvature, of uniform width to about the middle and then tapering to a sharp point. Apodeme short with a wide end-plate. Parameres narrow, strip-like, with blunt distal end and several short hairs.

Material examined

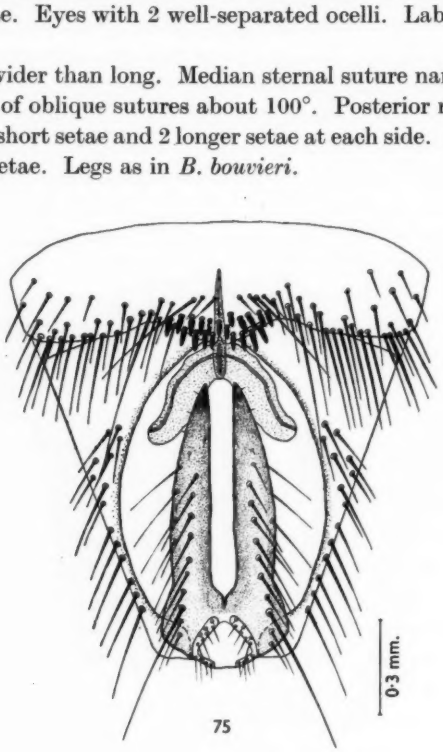
Type (♂) from 'West Africa', Muséum d'Histoire Naturelle, Paris. *Tanganyika*: Selya, Kondoa Irangi, 1 ♂, 1 ♀ (Zumpt). *Sudan*: El Obeid, *Scotophilus nigrita*, 1 ♀. *Sierra Leone*: Bonthe, *Scotophilus nigrita*, 1 ♀ (Bequaert). *Senegal*: Badi, *Scotophilus lencogaster*, *S. nigrita*, 1 ♂, 1 ♀ (Aellen); Magangani, Blue Nile, *Eptesicus phasma*, 1 ♀ (Phillips).

7. **Basilia (Paracyclopodia) glabra** n.sp.

Length 3 mm. Colour brown.

Head with a dense row of about 8 setae at the anterior dorsal margin and about 3 setae on the genae. Eyes with 2 well-separated ocelli. Labella of the labium as long as the theca.

Thorax slightly wider than long. Median sternal suture narrow, not widened in the middle. Angle of oblique sutures about 100°. Posterior margin of the sternal plate with a row of short setae and 2 longer setae at each side. A row of 9–12 rather short notopleural setae. Legs as in *B. bouvieri*.



Figs. 75, 76. *Basilia* (*Paracyclopodia*) *glabra* n.sp. 75, male, sternite 5 and genital area. 76, male, genitalia.

Abdomen, male (Fig. 75). Tergite 1 short with a dense double row of short setae at the posterior margin. Short spines in the middle of the surface. Tergites 2 and 3 with marginal rows of moderately long and short setae. A row of 5–6 short spines at the sides of the surface of tergite 2 and 2 short spines in the middle. Tergites 4–6

with longer setae. Surface of tergites 3–6 completely bare. There is no gap in the marginal row of tergite 6 and the long setae characteristic for the species of *Basilia* are absent. Anal segment conical, as long as the 3 preceding tergites, with moderately long setae laterally and posteriorly and some shorter setae in the posterior part of the dorsal surface. Sternite 1 + 2 rather short, trapezoid, with a ctenidium of 47 short teeth and 1 or 2 long setae at the lateral posterior corners. 4–6 rows of rather long spines in the posterior part of the surface. Sternites 3 and 4 narrow, with marginal rows of long and short setae. Short setae on the surface and 2 long setae near the middle in the posterior row of the surface on sternite 3. Sternite 5 not much longer than sternite 4, with a rounded bulge in the middle of the posterior margin which bears a double row of short, thick spines which does not extend laterally beyond the middle third of the sternite. There are 8 longer spines in the posterior row and 12 shorter spines in the anterior row. A dense double row of long and short setae in the lateral thirds of the posterior margin.

Genitalia (Fig. 76). Claspers long, straight, tapering to a long, pigmented point. A long seta dorsally near the base and a row of shorter setae on the dorsal surface in the basal two-thirds. Basal arc broad, rounded, with long anterior process. Phallobase concave dorsally, with 2 short setae near the base and 2 near the distal end. Aedeagus strongly sclerotized, curved in an S-curve, of nearly uniform width in the basal two-thirds and tapering to a long, pointed tip. Apodeme curved, with long, broad end-plate. Parameres narrow, nearly straight with a short distal point and a few short hairs.

Female unknown.

Material examined

Belgian Congo: Panga, Aruwimi province, 1 ♂ (type) (Schouteden), Muséum du Congo Belge, Tervueren.

8. **Basilia (Paracyclopodia) madagascarensis** n.sp.

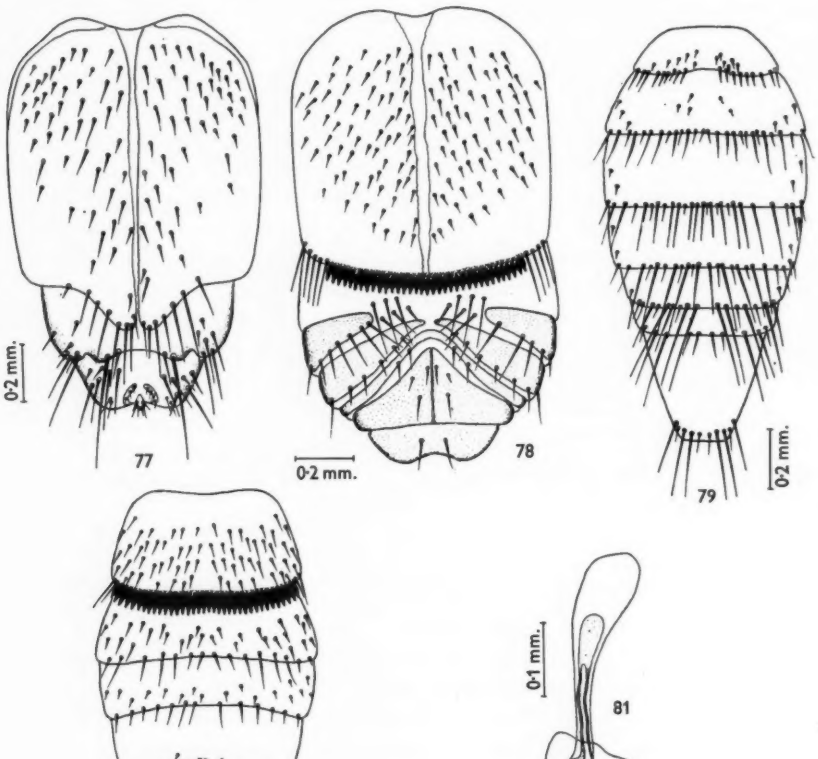
Length 2 mm. Colour yellowish brown.

Head. 4 setae at the anterior dorsal margin. Eyes with 2 well-separated ocelli. Labella of labium as long as the theca. Palps with short terminal seta.

Thorax slightly wider than long. Median sternal suture narrow. Angle of oblique sutures about 90°. Thoracic ctenidium with about 18 rather short teeth. Mesonotum narrow, lateral plates of the notopleural sutures wider behind the mesopleural suture. A row of 11–12 rather short notopleural setae. Tibiae very slender, 5 times as long as wide, tapering distally, with 3 rows of rather short setae in the middle. The distal row does not reach the tip of the tibia. Metatarsi very short.

Abdomen, female (Figs. 77, 78). Tergal plate 1 very long, rectangular, divided in the middle, with about 6 moderately long setae at the posterior processes and short spines on the surface. Tergal plate 2 more or less transversely rectangular with 2 thick and not very long setae and 2 short spines in the middle of the posterior margin and longer and shorter setae laterally. Anal segment short, rounded, with

a double row of setae around the anus. Sternite 1 + 2 very long, covering two-thirds of the ventral surface, with a ctenidium of 36 short, thick teeth which does not reach the sides and 3 or 4 setae at the lateral corners. Postspiracular sclerite narrow, curved, with 2–3 setae towards the spiracle. Surface covered with short spines except in the lateral posterior corners. Sternites 3 and 4 with triangular lateral sclerites which stand obliquely. Rows of setae at the posterior margin of the sclerites of sternites 3 and 4. Two groups of 6–8 moderately long setae between the sclerites of sternite 3. Sternite 5 strip-like, forming an angle with the apex anteriorly and with 3–4 setae at the posterior margin at each side. Sternite 6



Figs. 77–81. *Basilia* (*Paracyclopodia*) *madagascarensis* n.sp. 77, female, abdomen, dorsal. 78, female, abdomen, ventral. 79, male, abdomen, dorsal. 80, male, abdomen, ventral. 81, male, genitalia.

consisting of 2 triangular plates within the angle of sternite 5 and with several setae on the surface. Sternite 7 standing vertically to the ventral surface, rectangular, with some short setae posteriorly. Anal sclerite drop-shaped with several short setae.

Abdomen, male (Figs. 79, 80). Tergite 1 short, with short setae at the posterior margin and a gap in the middle of the row. Tergites 2 and 3 with marginal rows of short, widely spaced setae, tergites 4–6 with longer setae. Surface of tergites 2–6 bare, except for a few small hairs at the sides of the surface of tergites 2–4 and a few in the middle of tergite 2. Tergites 5 and 6 much shorter than the preceding tergites. Anal segment short, conical, bare dorsally. Sternite 1 + 2 short, with a ctenidium of 36 short, thick teeth and 1 or 2 setae laterally. Sternites 3 and 4 with marginal rows of widely spaced short setae. Sternite 5 similar, with straight posterior margin which bears an irregular double row of about 10 short, thick spines in the middle and setae laterally.

Genitalia (Fig. 81). Claspers thin, straight, with dark point and a long seta dorsally near the base. Phallobase short, concave dorsally, with 2 setae near the base and 2 further distally. Aedeagus nearly straight, of uniform width in the basal two-thirds and slightly upturned apical end. Apodeme short, with wide endplate. Parameres narrow, slightly curved, with blunt distal end.

Material examined

Madagascar: Nossi Bé, *Scotophilus borbonicus*, 1 ♀, 2 ♂ (types), British Museum (Nat. Hist.).

The specimens were not in a good state of preservation and not all details could be made out. The species resembles *B. roylii*, an Oriental species, in the female in the arrangement of the abdominal sclerites much more closely than the African species of the subgenus described above.

Genus **Tripselia** Scott, 1917

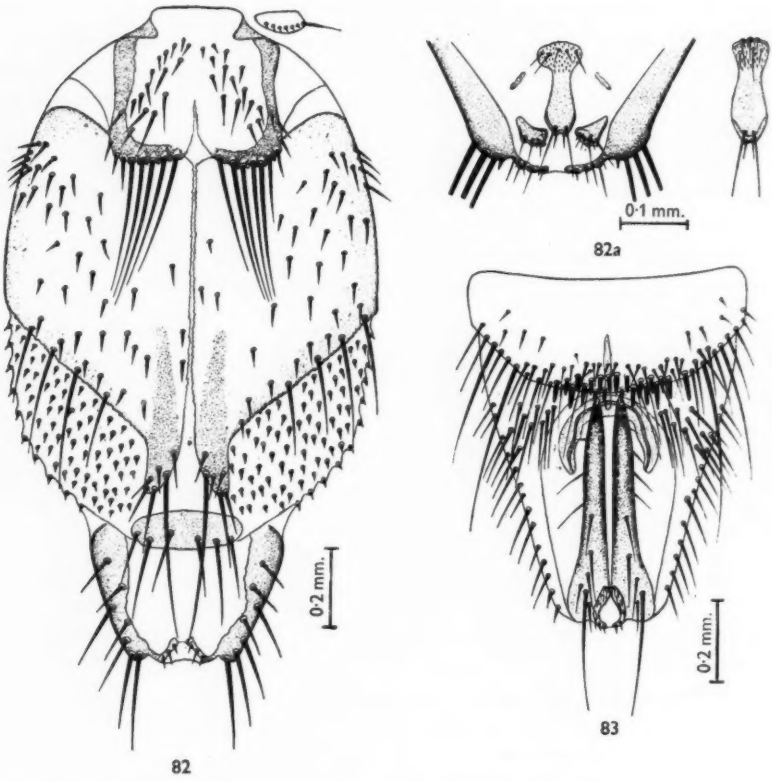
This genus has been discussed, a definition of the genus given and its species described in detail in a previous paper (Theodor, 1956). It has been shown that the genus contains a number of species, mainly in the Oriental Region and in Australia, and that it is closely related to some species of the genus *Basilia*, differing from them mainly in the absence of eyes. The length of the legs has been shown to be characteristic for 2 species only, and is apparently not a generic character.

Diagnosis. Head and *thorax* as in the genera *Nycteribia* and *Basilia*. Eyes absent. Legs either very long or of normal length. Tergite 1 of the female abdomen with 2 groups of long setae. Tergal plate 2 heart-shaped, with 2 posterior processes, as in the *Basilia bathybothyra* group. Genital plate and anal sclerite of female as in *Basilia*. Abdomen of male with 7 tergites with marginal rows of long setae and with short setae on the surface. Sternite 5 with an armature of spines at the posterior margin. Genitalia of *Basilia* type.

1. **Tripselia blainvillii** Leach, 1917

Syn. *Tripselia fryeri* Scott, 1917.
Nycteribia reichenowi Karaman, 1948.
Tripselia amiculata Speiser, 1907, apud Scott, 1925 pro parte.
nec *Nycteribia blainvillii* Leach apud Speiser, 1901.

For a full description the reader is referred to the above-mentioned paper (Theodor, 1956). The main characters are as follows:



Figs. 82, 83. *Tripselia blainvillii*. 82, female, abdomen, dorsal: *a*, genital plates and anal sclerite. 83, male, sternite 5 and genital area.

Thorax. Posterior margin of the sternal plate with a row of only very short hairs. Legs very long (3·5–4 mm.). Tibiae 8 times as long as wide with 3 rows of short setae in the middle of the ventral surface.

Abdomen, female (Fig. 82). Tergite 1 with 2 groups of about 5 long setae, all of about equal length. Tergal plate 2 heart-shaped, with long curved posterior processes which bear a long seta, a shorter one at the tip and several short spines. Tergal plate 3 transversely elliptical with several setae of moderate length. Abdominal

ctenidium with about 55 teeth. Sternites 5 and 6 with 2 lateral sclerites. Genital plate transversely elliptical, with several setae. Anal sclerite elliptical with 2 longer and 2 shorter setae.

Abdomen, male (Fig. 83). Tergites 2–6 with marginal rows of moderately long setae and 2–4 very long setae in the middle of the marginal rows of tergites 5 and 6. Small spines in the middle of the surface of the tergites which are more numerous on the anterior tergites. Sternite 5 with a double row of about 25 short spines at the posterior margin.

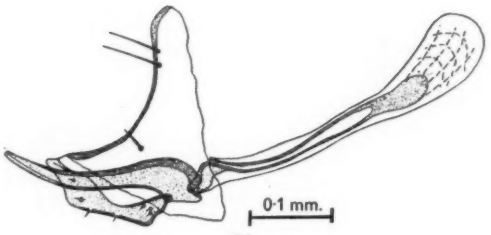


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Fig. 84. *Tripselia blainvillii*, male, genitalia.

Genitalia (Fig. 84). Aedeagus slightly curved, tapering to a point. Parameres with blunt tip and obtuse ventral angle.

Distribution. Mauritius, Assumption Islands, Madagascar. Tropical Africa from west to east between 10° lat. north and south.

Host. Mainly *Taphozous mauritianus.*

Full details of material examined, localities and hosts have been given previously (Theodor, 1956).

2. **Tripselia aequisetosa** Theodor, 1956

This species resembles *T. blainvillii* closely. It differs from it in the presence of some longer setae at the posterior margin of the sternal plate of the thorax and in the chaetotaxy of the female abdomen. There are only 2–3 long setae on tergite 1 and the innermost and the lateral setae are shorter. The posterior processes of tergal plate 2 are broader and bear only 6–9 short spines and no long setae (Fig. 85).

Distribution. Nigeria, Liberia, Belgian Congo.

Host. Saccolaimus peli.

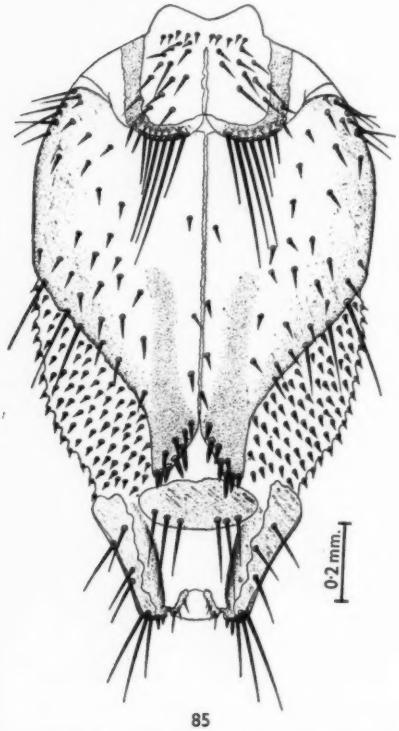
3. **Tripselia echinata** n.sp.

Length 3 mm. Colour brown.

Head laterally compressed. The anterior margin forms a sharp angle, each side bearing 3 setae near the apex, and 2 more setae inside the angle. Eyes absent. Anterior ventral margin with a row of short setae. Labella of the labium about one third of the length of the theca.

Thorax wider than long, rounded anteriorly. Length to width = 4:5. Median

ventral suture narrow. Oblique sutures forming an angle of about 100°. The posterior ventral margin bears a row of longer setae laterally and shorter setae near the middle. Thoracic ctenidium with 21–22 long, pointed teeth. Mesonotum slightly wider posteriorly, with a rather high posterior plate. 8–10 notopleural setae. Legs rather short, tibiae scalpel-shaped, 4 times as long as wide, with 3 rows of setae in the distal half. The setae of the distal row are the longest and reach beyond the tip of the tibia (Fig. 86).



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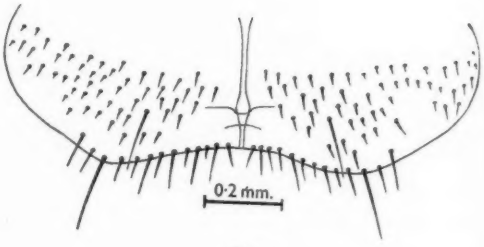
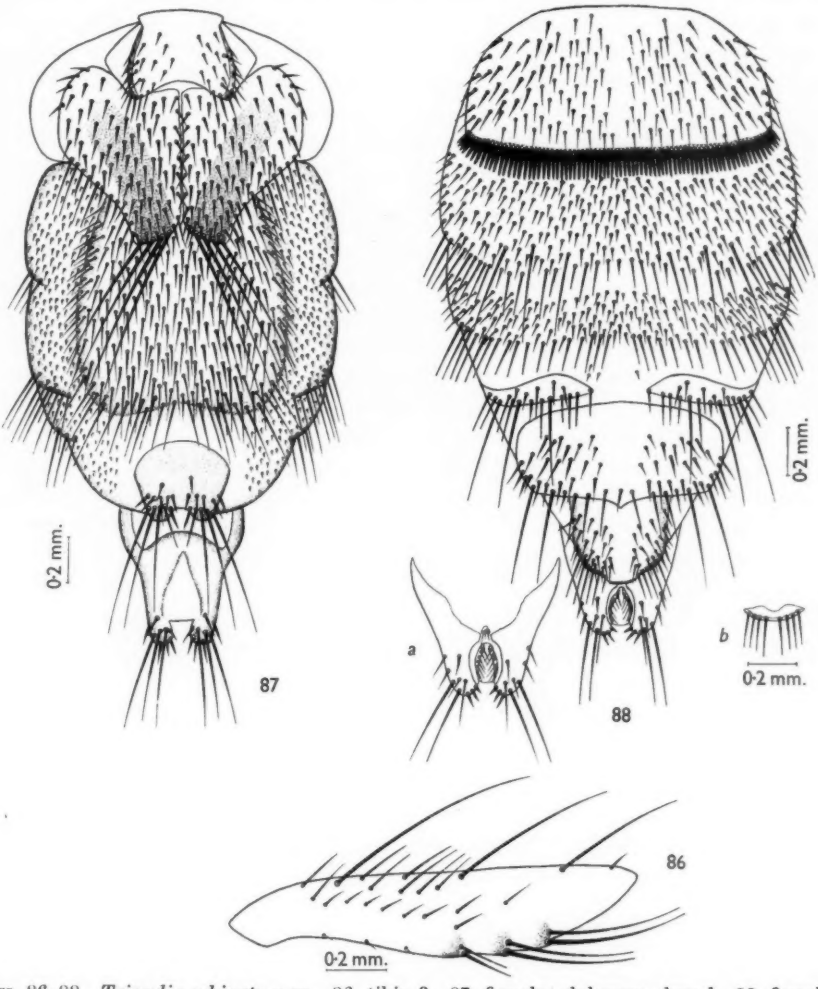


85 a

Fig. 85. *Tripselia aequisetosa*, female, abdomen, dorsal: *a*, posterior ventral margin of thorax.

Abdomen, female (Figs. 87, 88). Tergite 1 with a concave posterior margin which bears 4 short spines at each lateral corner. The middle of the margin is bare. Surface laterally covered with short spines. Tergal plate 2 very short, wider than long, with lateral rounded flaps, divided in the middle. There are 3–4 very long setae at the posterior processes. A dense row of short setae along the posterior lateral margins. The whole surface is thickly covered with short spines, which are longer posteriorly. A broad, pigmented stripe runs from the posterior process into the middle of each half of the surface. The membrane between tergal plates 2 and 3 is also thickly covered with short setae anteriorly and longer setae posteriorly. Tergal plate 3 trapezoid, with 2 rounded processes posteriorly which bear 3 long setae and about 8 short spines. 2 short spines in the middle of the surface. Anal segment conical,

bare dorsally and laterally with long anal processes, which bear 4–5 long setae and some short spines. Pleurae covered with minute spines. Sternite 1 + 2 short and wide, with a ctenidium of about 80 narrow teeth. Surface covered with short spines. Postspiracular sclerite with 3 long setae towards the spiracle. Sternite 3 with a marginal row of shorter setae in the middle and longer setae laterally.



Figs. 86–88. *Tripselia echinata* n.sp. 86, tibia 3. 87, female, abdomen, dorsal. 88, female, abdomen, ventral: *a*, anal segment ventral, with anal sclerite; *b*, genital plate.

There is a premarginal row of 6 long setae. The surface is covered by 6–8 rows of short setae which become gradually longer laterally and posteriorly. Sternite 4 similar but with only 3–4 rows of short setae in the middle and 5–6 rows laterally. The premarginal setae are absent. Sternite 5 divided into 2 narrow elliptical sclerites which bear 3 long setae and some shorter setae on the posterior margin and some

short setae on the surface. Sternite 6 undivided, its posterior margin forming an obtuse angle which bears some long setae laterally and shorter setae towards the apex. About 3 rows of short setae on the surface. Sternite 7 much narrower than sternite 6, about as long as wide, rounded posteriorly. A dense row of short setae at the posterior margin and 2 groups of short setae in the posterior lateral parts of the surface. Dorsal genital plate narrow, curved, with 7 setae. Anal sclerite small, with 3 short setae.

Male unknown.

Material examined

Southern Nigeria: Old Calabar, *Eptesicus brunneus*, 1 ♀ (type), British Museum (Nat. Hist.).

Genus **Penicillidia** Kolenati, 1863

Diagnosis. Medium- or large-sized insects, generally very setose. *Head* broadly rounded, not laterally compressed, with single, unpigmented eyes. Palpi thick, with several rows of setae at the ventral surface. *Thorax* broader than long. Mesonotum narrowing posteriorly. Notopleural setae either numerous, few or absent. Haltere groove cover present. Thoracic ctenidium with long, pointed teeth. Oblique sutures indistinct. Legs long, tibiae slender, scalpel-shaped or parallel-sided with several rows of setae distally. Abdominal ctenidium either normal or reduced, with spines which are shorter and more widely spaced than in other genera. Postspiracular sclerite absent. 2 genital plates in the female, of which the dorsal may bear setae. Male genitalia of *Nycteribia* type. Sternite 5 of male abdomen with several rows or groups of spines at the posterior margin.

Two species occur in Africa and 2 others in Madagascar. *Penicillidia senegalensis* Gervais has to be considered as a *nomen nudum* as no description has been published and the drawing given by Gervais does not permit identification.

Key to the species of Penicillidia *of the Ethiopian Region and Madagascar*

1. Small, not very setose species (2·5–3 mm.). Tergal plate 3 of female abdomen divided into 2 widely separated sclerites. The group of spines at the posterior margin of sternite 5 of the male abdomen consists of a posterior row of about 18 longer spines and an anterior row of 8–10 shorter spines. Aedeagus with a subapical tooth. Madagascar (Figs. 102, 104, 105). **Penicillidia leptothrinax** Speiser

— Large, setose insects (4–5 mm.). 2

2. 5–6 long notopleural setae present. Tergal plate 3 of female abdomen absent. Madagascar (Figs. 94, 95). **Penicillidia decipiens** n.sp.

— Notopleural setae absent. Tergal plate 3 of female abdomen present or indicated by groups of setae. 3

3. Sternite 1 + 2 as wide as abdomen, with a ctenidium of very short, widely spaced spines. Tergal plate 3 of female abdomen broad, undivided. Armature of spines at the posterior margin of sternite 5 of the male consisting of a double row of 40–50 spines. Femora 2 and 3 with long setae only in the distal part of the anterior surface. Setae of the distal row on the tibiae longer than the width of the tibia (Figs. 90, 92, 96). **Penicillidia fulvida** Bigot

— Sternite 1 + 2 narrower than the abdomen, with longer spines in the ctenidium. Tergal plate 3 of female consisting of 2 small sclerites or only of groups of setae.

Armature of spines at the posterior margin of sternite 5 of the male consisting of 60–70 short spines in 3 rows. Femora 2 and 3 covered with moderately long setae on the whole anterior surface. Setae of the distal row on the tibiae not longer than the width of the tibia (Figs. 96, 97, 99). **Penicillidia pachymela** Speiser

1. **Penicillidia fulvida** Bigot, 1885

Falcoz (1924) has given a description of the female and both sexes have been described by Theodor (1954). The main characters are as follows:

Size 4–5 mm. Colour reddish brown. *Thorax* (Fig. 89) markedly wider than long. Notopleural setae absent. Oblique sutures fused, forming an angle of about 100°. Femora twice as thick as the tibiae. Femur 1 covered with long setae in more than the distal half, femora 2 and 3 only in the distal third of the anterior surface. Tibiae slender, with 4 rows of long setae in the distal part of the ventral surface. The setae of the distal rows are longer than the width of the tibia and reach beyond the tip (Fig. 96).

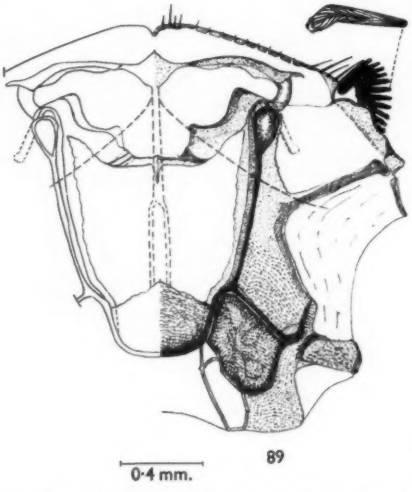
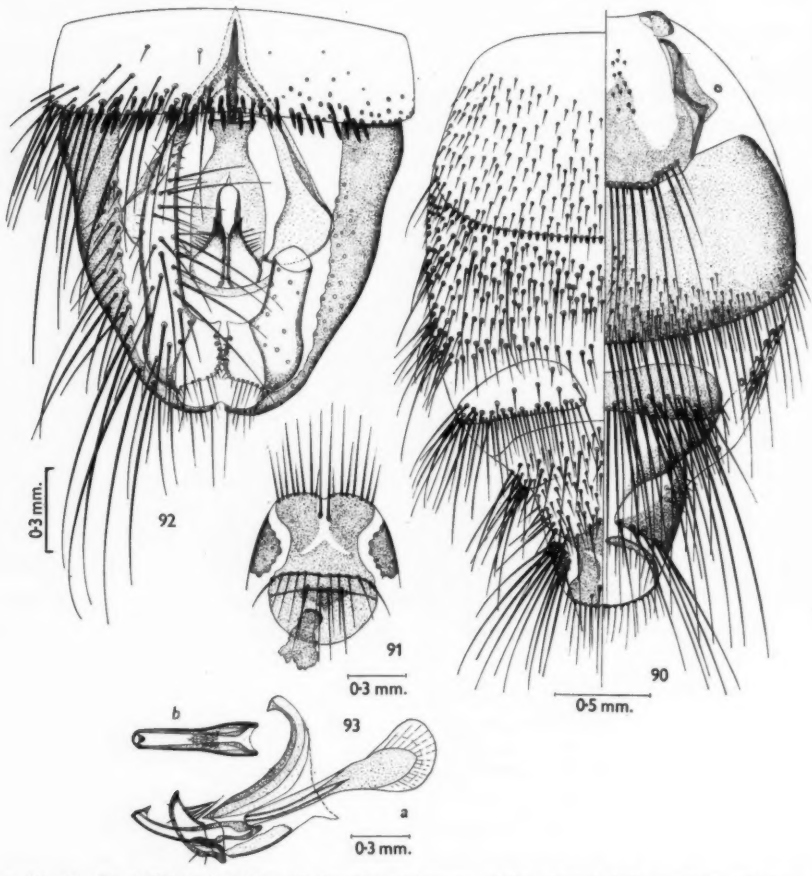


Fig. 89. *Penicillidia fulvida*, dorsal pattern of thorax.

Abdomen, female (Fig. 90). Tergite 1 pentagonal, with a row of about 20 long setae posteriorly. Tergal plate 2 with a single or double row of long setae posteriorly and short hairs on the posterior part of the surface. Tergal plate 3 short and wide, undivided, wider laterally and with a double or triple row of long setae posteriorly. In some specimens tergal plate 3 is divided into 2 lateral, elliptical sclerites. Abdomen bare between tergal plates 2 and 3 and between tergal plate 3 and the anal segment. Sternite 1 + 2 as broad as the abdomen, with a ctenidium of 50–55 very short, ordinary spines which do not stand as closely as in other genera. Sternite 5 with 2 broad, elliptical, obliquely standing sclerites with long setae posteriorly. Dorsal genital plate (Fig. 91) broadly rounded, covering the genital opening from above, with a double row of 12–15 long and short setae near the base.

Abdomen, male (Fig. 92). Tergites with dense double rows of long setae posteriorly and short hairs on the surface. Anal segment narrow, with a deep concavity at the anterior dorsal margin. Sternite 5 with a double row of 40–50 short spines. The lateral spines of the posterior row are longer than the median ones and those of the anterior row.



Figs. 90–93. *Penicillidia fulvida*. 90, female, abdomen: right, dorsal; left, ventral. 91, female, genital plates. 92, male, sternite 5 and genital area. 93, male, genitalia: *a*, profile; *b*, aedeagus, dorsal.

Genitalia (Fig. 93). Claspers slightly curved, with long pigmented tip, densely covered with long setae in the basal two-thirds of the dorsal surface. Basal arc triangular, with long anterior process. Aedeagus slightly curved, tapering, with a long apical tooth and a few rows of scale-like teeth in the middle of the ventral surface. Parameres triangular, with a row of 5–6 setae at the dorsal edge.

Material examined

South Africa. *Cape Province*: Knysna, *Eidolon helvum*, 1 ♀ (Rothschild collection). *Natal*: Pietermaritzburg district, *Rhinolophus clivosus augur*, 17 spec., *R. clivosus zuluensis*, 1 ♂, *Miniopterus schreibersi natalensis*, 9 spec., *Myotis tricolor*, 2 spec., *Nycteris capensis*, 3 spec. (Lawrence). *N. Transvaal*: Makapan caves, Potgietersrust, *Miniopterus schreibersi natalensis*, 5 spec. (Zumpt). *Transvaal*: Sterkfontein near Kruegersdorp, *Rhinolophus clivosus augur*, 1 ♀ (Zumpt).

Belgian Congo. Kivu, Rutshuru, 2 spec.; Thysville, *Miniopterus inflatus*, 3 spec. (Bequaert); Panga, Aruwimi district, 1 ♀ (Schouteden); Yolohafiri cave, Irumu district, 4 spec. (Leleup); Kakontwe, Katanga, 1 ♀ (Leleup).

West Africa. Mt Cameroons, *Miniopterus inflatus*, 1 ♀ (Boulton), Musake: Mt Cameroons, *Miniopterus inflatus*, 3 spec. (Eisentraut). *Cameroons*: Buea, *M. inflatus*, 3 spec. (Eisentraut); Karba Manga, *Rhinolophus foxi*, 2 spec. (Aellen).

East Africa. *Tanganyika*: Magrotto near Tanga, *Rhinolophus hildebrandti*, 1 ♂ (Loveridge). *Kenya*: Kericho, *Hipposideros caffer*, 1 ♀ (Dobbs); Mt Elgon, *Miniopterus schreibersi arenarius*, 21 spec., *Rhinolophus clivosus keniensis*, 1 ♀ (Edwards); Mt Menengai, Rift Valley, *Miniopterus schreibersi arenarius*, *Myotis tricolor*, 10 spec. (Hoogstraal). *Sudan*: Torit district, Equatoria province, *Miniopterus* sp., *Coleura gallarum*, 2 spec. (Owen). *Sudan*: *Rhinolophus eloquens*, 1 ♂ (Hoogstraal).

Arabia. *Yemen*: San'a, *Rhinolophus blasii*, 1 ♀ (Hoogstraal).

The single female from Arabia differs in some details from the African specimens. Tergal plate 3 is divided into 2 sclerites which are smaller and bear less setae than in the African specimens and the genital plate is narrower and longer. Whether these differences are constant and perhaps of subspecific value will have to be determined by examination of more material.

2. **Penicillidia decipiens** n.sp.

Syn. *Penicillidia fulvida* Bigot? (pro parte) apud Speiser, 1901

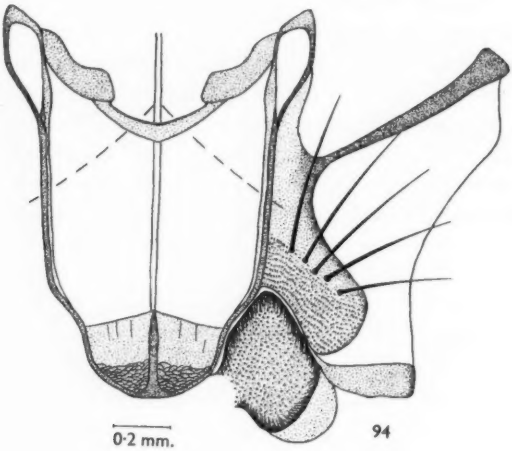
Speiser identified a male from Madagascar as *P. fulvida*. No specimens of *P. fulvida* from Madagascar have been examined and it therefore seems possible that the specimen examined by Speiser belonged to the new species described here which resembles *P. fulvida* closely in habitus.

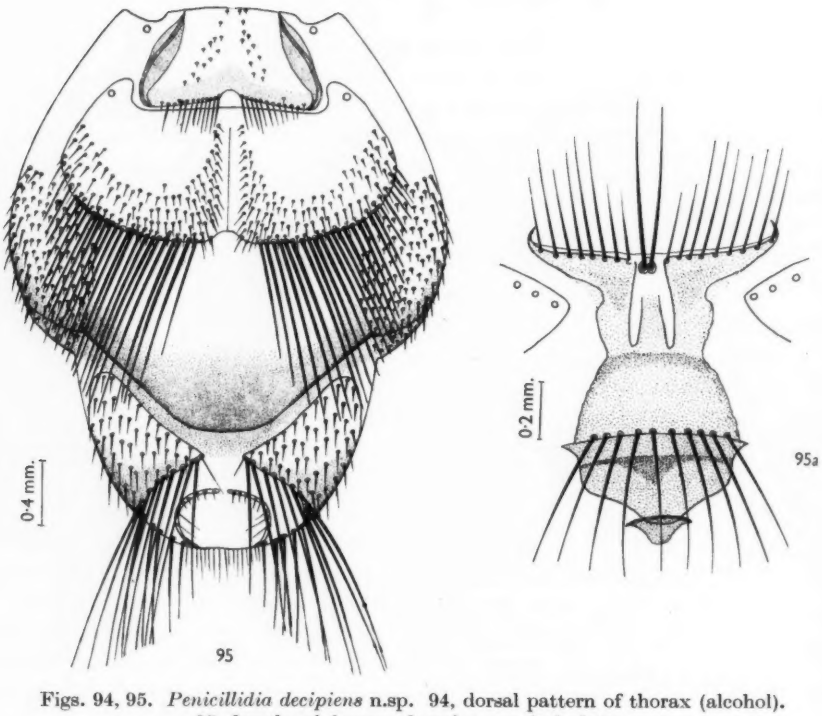
Length 4 mm. Colour reddish brown.

Head as in *P. fulvida*.

Thorax (Fig. 94) also as in *P. fulvida*, but differing in the following characters. There is a row of 5–6 very long notopleural setae, which are absent in *P. fulvida*. The lateral plates of the notopleural sutures are much narrower anterior to the mesopleural suture than posterior to it. (They are less wide posteriorly in *P. fulvida*.) The small sclerite between haltere groove and coxa 3 is narrow and parallel-sided, while in *P. fulvida* it is broader, with a rounded anterior margin. The setae on the anterior surface of the femora are shorter than in *P. fulvida* and cover the distal half of femur 1 and the distal third of femora 2 and 3.

Abdomen, female (Fig. 95). Tergite 1 triangular, with a marginal row of short setae with a gap in the middle. Tergal plate 2 broadly rounded with a dense marginal row of long setae with a gap in the middle, covered with short hairs in the posterior part of the surface and with a clearly marked, narrow, bare median stripe.





Figs. 94, 95. *Penicillidia decipiens* n.sp. 94, dorsal pattern of thorax (alcohol). 95, female, abdomen, dorsal: *a*, genital plates.

Tergal plate 3 is absent and the dorsum is bare between tergal plate 2 and the anal segment. The latter is large and bears a dense row of very long setae around the anus. The lateral sclerites of the anal segment are covered with short hairs. (There are only marginal rows in *P. fulvida*.) The short hairs on the pleurae reach farther dorsally than in *P. fulvida*. They reach beyond the spiracles anteriorly and up to the spiracles posteriorly, while in *P. fulvida* spiracles 4–6 lie in a completely bare area. Ventral surface as in *P. fulvida*, but sternite 1+2 is narrower and has a ctenidium of only 36 short and widely spaced teeth. The surface of the lateral sclerites of sternite 5 is nearly completely covered with short setae while there are only 1–2 rows of longer setae in *P. fulvida*. Dorsal genital plate as in *P. fulvida* but with only a single row of long, thick setae across the base. (2 to 3 rows of shorter setae in *P. fulvida*.) Ventral plate triangular.

Male unknown.

Material examined

Madagascar: Namaroka, Ambohimirija caves, 2 ♀ types (Paulian); Fort Carnot, 1 ♀.

3. **Penicillidia pachymela** Speiser, 1901

Speiser's short description from 2 males mentions the dark colour and the chaetotaxy of the legs as different from *P. fulvida*. Karaman (1948) gives a short description of the male and of the aedeagus. The female has not been described previously.

Length 4·5–5 mm. Colour: some specimens are blackish brown, while others have the same reddish brown colour as *P. fulvida*.

Head broadly rounded with small, single, unpigmented ocelli. A group of 5–6 dense rows of long setae between the eyes up to the anterior margin. Labella of labium shorter than the theca.

Thorax markedly wider than long. Length to width = 5:6·5. Median sternal suture narrow. Oblique sutures fused, forming an angle of about 100°. Thoracic ctenidia with about 22 long, pointed teeth. Mesonotum broad, parallel-sided, with broad posterior dorsal plate. Lateral plates of notopleural sutures moderately broad, with concave lateral margin. Notopleural setae absent. Femora thick, but not thicker than in *P. fulvida*. Anterior surface of femora covered with short setae up to the basal ring. These setae are longer distally. Tibiae (Fig. 96*a*) parallel-sided, with 5 rows of setae in the distal half of the ventral surface. The setae of the 2 distal rows are about as long as the width of the tibiae, very thick, and do not reach much beyond the tip of the tibiae. The setae of the basal rows are much thinner and shorter. (There are only 4 rows of much longer setae in *P. fulvida*.)

Abdomen, female (Figs. 97, 98). Tergite 1 rounded posteriorly with a marginal row of 25–30 setae. There is a premarginal row of similar setae in some specimens. Tergal plate 2 broadly rounded with a dense marginal row of long setae. Surface covered with short hairs in the posterior part which leave a median stripe bare. In some specimens this bare stripe is absent. Tergite 3 membranous with a marginal row of long setae, which are shorter than those of tergal plate 2. There are

2–3 rows of short setae in the middle of the surface and 4–5 rows laterally. Tergite 4 with 2 groups of long setae with a bare gap between them. These groups consist of a double row of long setae posteriorly and shorter setae anteriorly. In some specimens the groups are not clearly marked and the setae are more widely spaced. In others the groups of setae stand on not clearly outlined sclerotized areas. Setae of the surface as on tergite 3. The chaetotaxy of the dorsum of the abdomen is extremely variable so that in some specimens the middle of the abdomen is bare, while in others nearly the whole dorsum is covered with equally spaced setae and there is only a small bare area in the middle. Anal segment rounded, sclerotized, with a row of long setae surrounding the anus and shorter setae anteriorly. Anal frame circular, narrow dorsally and widened ventrally. Pleurae covered with short

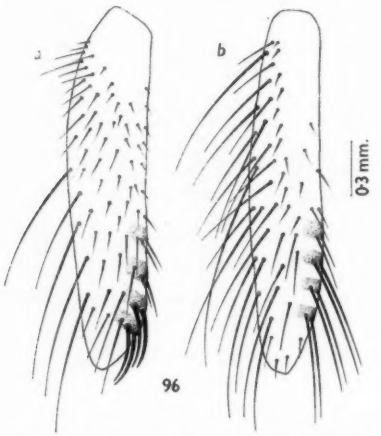
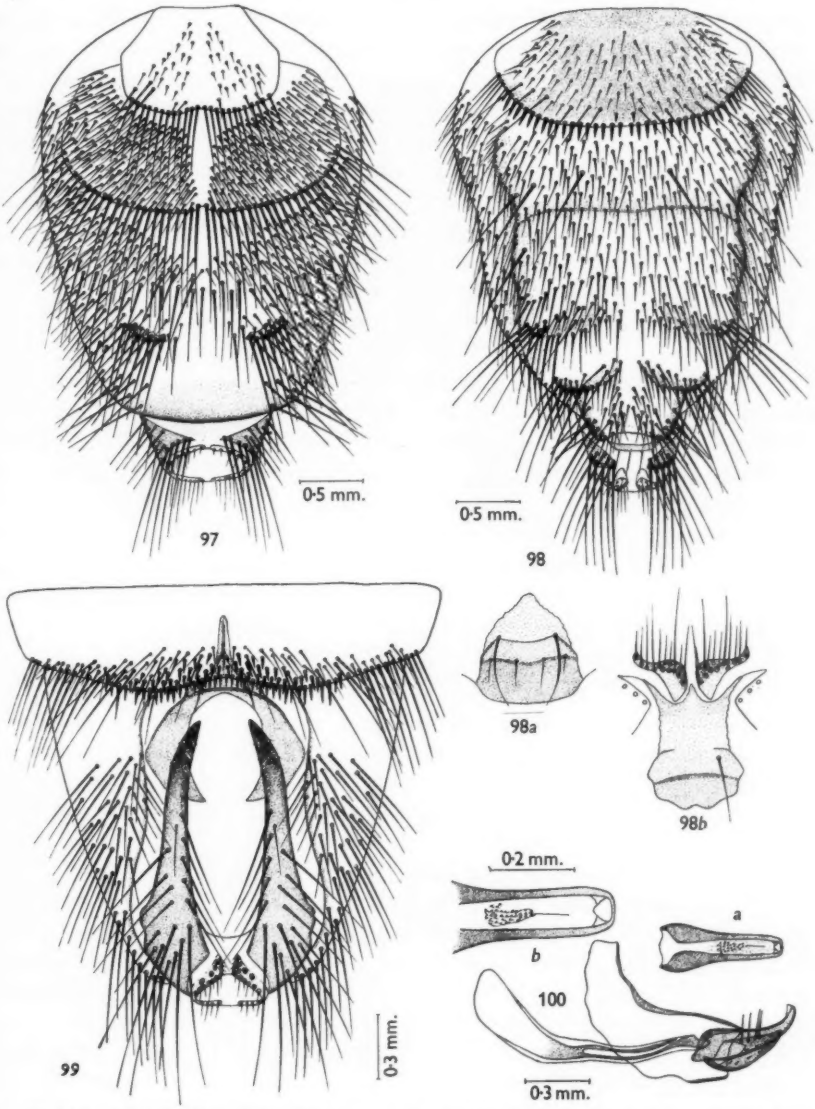


Fig. 96. Tibia 3 of *a*, *Penicillidia pachymela*; and *b*, *P. fulvida*.

setae. Sternite 1+2 narrower than the abdomen, rectangular, with a ctenidium of about 40 widely spaced short spines which are longer laterally. 7–8 rows of short setae on the surface. The posterior row of the surface consists of a row of long setae and short setae between them. Sternite 3 with a marginal row of short setae. Surface covered with 8–9 rows of short hairs and an irregular row of 6–10 long setae in the middle. Sternite 4 similar but narrower. The marginal setae are longer and there are only 4–5 rows of short hairs on the surface. Only 2–4 long setae among the short hairs of the surface. Sternite 5 is indicated by 2 obliquely standing groups of setae which stand on not clearly defined sclerotized areas in some specimens. These groups consist of 3–5 rows of setae which are longer laterally and posteriorly. The posterior margin of the sternite projects above the surface. Sternite 6 similar but the groups of setae stand more closely together. Sternite 7 with 2 rounded lobes posteriorly which bear long and short setae and 2–3 rows of short setae on the surface in the posterior part. Dorsal genital plate large, semicircular, covering the genital opening from above. There is a transverse row of 2 long setae near the base and 4 shorter setae in some specimens and one or none in others (Fig. 98*a*, *b*). Ventral genital plate absent.

Abdomen, male (Fig. 99). Tergite 1 as in the female. Tergite 2 also similar but much shorter. Tergites 3–6 narrow, with dense, irregular, double rows of long and short setae at the posterior margin. Tergite 3 covered with short setae on the surface except for a median stripe. Only a single row on the surface of tergite 4 and tergites 5 and 6 are bare. Anal segment broadly truncate with deeply concave



Figs. 97–100. *Penicillidia pachymela*. 97, female, abdomen, dorsal. 98, female, abdomen, ventral: *a*, genital plates with setae; *b*, with one seta. 99, male, sternite 5 and genital area. 100, male, genitalia: *a*, *b*, aedeagus, dorsal.

anterior dorsal margin, bare on the dorsal surface and with some long setae posteriorly and laterally. Sternite 1 + 2 as in the female. Sternites 3 and 4 narrow with rather short setae posteriorly and 1–2 rows of short setae on the surface. Sternite 5 as long as sternites 3 and 4 together, rectangular, with 60–70 short spines in about 3 rows along the posterior margin. The lateral spines are longer. The rows of spines do not reach the lateral corners where there are only long and short setae. Some short setae anterior to the spines.

Genitalia (Fig. 100). Claspers strong, curved, tapering to darkly pigmented tips. Dorsal surface with several rows of long setae in the basal two thirds. These setae are less numerous and shorter than in *P. fulvida*. Basal arc with broad lateral flaps and long anterior process. Phallobase broad, concave dorsally, dorsoventrally depressed, with 2 pigmented stripes which unite at the base. No setae near the base. Aedeagus tapering, curved, with a short dorsal tooth at the tip and a small group of scale-like teeth at the ventral surface. Apodeme long with narrow end-plate. Parameres triangular with dark tips. A row of 4–5 setae in the middle of the dorsal margin and some minute hairs at the ventral edge.

Distribution. Tropical Africa from Somaliland to Cameroons between 10° lat. north and south.

Two females from the Cameroons differ from the form described in a number of details. Femora and tibiae are thicker and shorter. Tergal plate 2 is completely covered with short hairs without a bare median stripe. The dorsum of the abdomen is completely covered with short setae on tergites 3 and 4 without any median bare area. There is only a small bare patch in front of the anal segment. The setae on tergite 4 are evenly distributed across the dorsum and do not form groups. Some of these characters have been observed also in specimens from other localities. They do not seem to be restricted geographically and are so variable that much more material is needed before local forms or subspecies can be defined.

Material examined

EAST AFRICA. *Sudan*: Equatoria province, Katire near Torit, *Rhinolophus eloquens* or *R. landeri lobatus*, 1 ♀ (Hoogstraal). *Kenya*: Nairobi, 3 spec. (van Someren). *Tanganyika*: Banaqui Hill, 3 spec.; Kilimafesa, Serengeti, *Nycteris* sp., 2 spec.; Kilosa, *Hipposideros caffer*, 2 spec. (Loveridge); Rukwa valley, *H. caffer*, 1 ♀ (Robertson).

BELGIAN CONGO. Kakontwe, Katanga, 1 ♂ (Leleup); Mulungwisi, Katanga, 2 spec. (Bequaert); Gabon, La Bamba, Bongola, *Hipposideros caffer*, 1 ♀ (Beatty); Pelenge, Kiwakishi, Masombwe, Mabwe near Lake Upemba, *H. caffer*, 6 spec. (Sundeval).

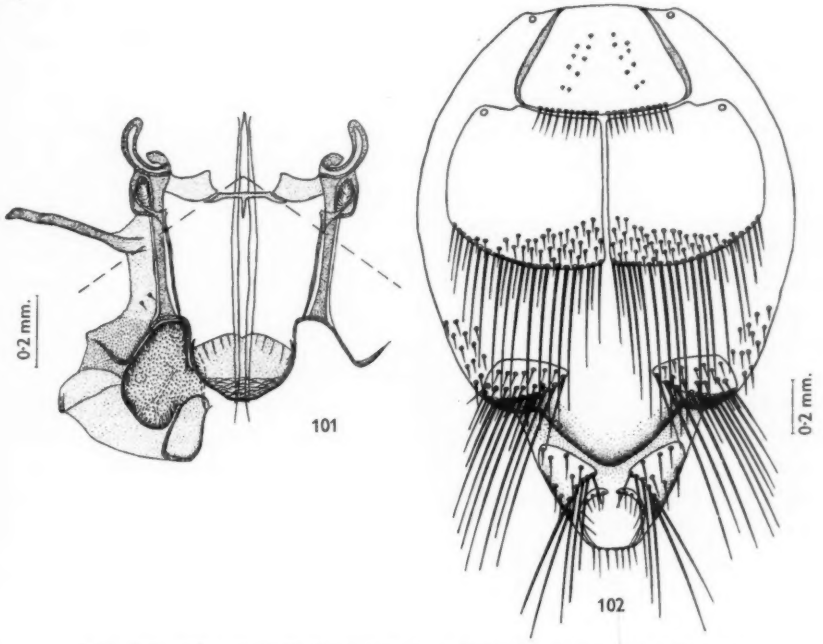
FRENCH EQUATORIAL AFRICA. Guembe, 4 spec. (very dark brown specimens), (Grjebine).

WEST AFRICA. *Cameroons*: Nyassoso, *Hipposideros* sp., 1 ♀ (Eisentraut); Buea, *Rhinolophus landeri*, 1 ♀ (Eisentraut).

4. **Penicillidia leptothrinax** Speiser, 1908

Length 2·5–3 mm. Colour yellowish brown, head darker brown.

Head broadly rounded, with rather large, unpigmented, single eyes. About 5 rows of setae between the eyes. A row of short setae with a long seta in the middle at the anterior ventral margin and 2–3 rows of short spines near the marginal row. Labella of the labium about half as long as the theca. Terminal seta of palpi very long.



Figs. 101, 102. *Penicillidia leptothrinax*. 101, dorsal pattern of thorax. 102, female, abdomen, dorsal.

Thorax (Fig. 101) markedly wider than long. Length to width = 3:4. Anterior margin of the sternal plate produced in the middle. Sternal plate strongly convex in the longitudinal direction, as in *P. jenynsii*. Median sternal suture narrow. Oblique sutures scarcely visible, forming an angle of about 95°. Posterior ventral margin straight, with a row of long and short setae and a long seta at each side. Mesonotum narrowing posteriorly. Anterior spiracles large and elliptical. Lateral plates of the notopleural sutures narrow with concave lateral margin. 2–3 short and thin notopleural setae. Legs moderately long, femora not very thick. Anterior surface of femur 1 covered with moderately long setae in the distal two-thirds, femur 2 and 3 with only 2–3 rows of setae near the distal end. Tibiae slender, parallel-sided, with 3–4 rows of setae near the distal end. Those of the 2 apical rows are long and thick and those of the basal rows short and thin.

Abdomen, female (Figs. 102, 103). Tergite 1 trapezoid, with slightly rounded

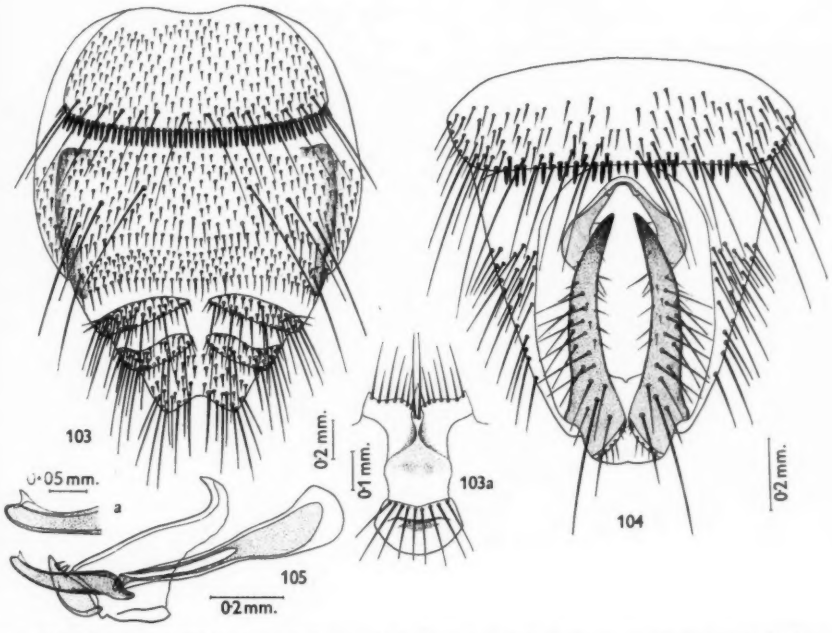
posterior margin which bears a row of 14–20 short spines which does not reach the sides. In one specimen only 12 spines with a large gap in the middle, in two others about 20 spines without a gap. Tergal plate 2 broad, rectangular, with a median division line. The marginal row consists of very long setae alternating with short spines and a few shorter setae in the middle. Several rows of short spines anterior to the marginal row, 4–5 rows in the middle and 2–3 laterally. Tergal plate 3 divided into 2 widely separated, elliptical or triangular sclerites. They bear a row of long setae at the posterior margin, a premarginal row of long spines and 1 or 2 rows of short spines on the surface. Anal segment short, conical with a row of long setae surrounding the anus and short spines on the surface. Dorsum of abdomen bare between tergal plates 2 and 3 and between tergal plate 3 and anal segment. Pleurae covered with short spines which are longer posteriorly. Sternite 1+2 trapezoid, with a ctenidium of about 50 closely standing teeth and 2–3 long setae at the lateral corners. Surface covered with short spines. A row of about 15 long setae in the posterior row of the surface. Sternites 3 and 4 with marginal rows of short setae which are not much longer than the spines of the surface in the middle of the marginal row of sternite 3 and longer laterally. The marginal setae of sternite 4 are about twice as long as those of the surface. A curved row of 6 long setae on the surface of sternite 3. A long seta on the surface of sternite 4 near the lateral posterior corners. Sternites 5 and 6 consist of narrow elliptical sclerites, which stand obliquely and project from the surface posteriorly. They bear long setae posteriorly and laterally and short spines on the surface. Sternite 7 with a deep concavity posteriorly, with long setae posteriorly and short setae on the surface. Dorsal genital plate large, semicircular, forming a lip which covers the genital opening from above. A row of about 10 long setae at the base. Ventral plate small, unarmed, triangular.

Abdomen, male (Fig. 104). Tergite 1 as in the female, but with a row of 10–12 longer setae in the middle of the posterior margin which are longer and thinner than the spines of the female in this position in some specimens but rather short and thin in others. Tergite 2 with a marginal row of moderately long setae. Tergites 3–6 with marginal rows of very long and shorter setae. Surface of tergites 2–6 covered with short setae. Tergite 3 is the longest, tergites 4 and 5 are shorter and 5 and 6 are narrow, strip-like, with concave anterior margin. Anal segment narrow, conical, with concave anterior dorsal margin and a row of moderately long setae around the anus Anterior to this row another row of 6–8 very long setae. Short setae in the posterior part of the surface and at the sides. Sternite 1+2 as in the female. Sternites 3 and 4 narrow, with short setae on the surface and a marginal row of long and short setae. The lateral setae are longer than the median ones. Sternite 5 longer, with a similar marginal row of setae and a double row of spines in the middle of the posterior margin. About 15–20 longer spines in the posterior row and about 10 shorter spines alternating with long setae in the anterior row. Surface covered with short setae in the posterior two-thirds.

Genitalia (Fig. 105). Basal arc with moderately wide lateral flaps and without anterior process. Claspers curved, with dark blunt tips. A long seta dorsally near the base and several rows of shorter setae in the basal two-thirds. Phallobase con-

cave dorsally, dorsoventrally depressed. Aedeagus slightly curved, tapering, with a blunt tip and a long subapical tooth. An area of scale-like teeth at the ventral surface. Apodeme with a wide end-plate. Parameres triangular, with a row of 4–5 setae at the dorsal margin and some short hairs at the curved ventral margin.

P. leptothrinax resembles the Oriental *P. jenynsii* in size, the shape and curvature of the sternal plate of the thorax, arrangement of the abdominal sclerites in the female and a number of other characters.



Figs. 103–105. *Penicillidia leptothrinax*. 103, female, abdomen, ventral: *a*, genital plates. 104, male, sternite 5 and genital area. 105, male, genitalia: *a*, tip of aedeagus.

Material examined

Madagascar: Nossi Bé, *Miniopterus schreibersi*, 1 ♂; Ambohinatombo, *Myotis goudoti*, 2 ♀ (Major); *Miniopterus manavi*, 1 ♂; Andranoboka, Tananariva, Majunga road, 1 ♂ (Tipton); Namoroka, Ambohimiriya caves, *Myotis goudoti* or *Pipistrellus nanus*, 1 ♀ (Paulian); Imasindrary, N.E. Betsileo, *Miniopterus schreibersi natalensis*. (*M. scotinus*), 1 ♀, 1 ♂ (Major).

Genus **Eucampsipoda** Kolenati, 1857

The genus has been revised and the African and Madagascar species have been described in detail in a previous paper (Theodor, 1955).

Diagnosis. Head laterally compressed, pigmented dorsally, with elliptical, single, unpigmented eyes. Palps finger-shaped, with a thick base. Labella of

labium longer than the theca. Thorax pentagonal, wider posteriorly. Thoracic ctenidia consisting of thick, blunt spines. Mesonotum narrow, widening posteriorly. One long and one short notopleural seta. Haltere groove without cover. Oblique sutures of the sternal plate well marked. Tibiae long, cylindrical, parallel-sided, with 2 rings and short setae in the middle. Tergites 1 and 2 of abdomen fused. Abdominal ctenidium consisting of long, thick and blunt spines. Postspiracular sclerite with a single seta. Segmentation of the abdomen of the female reduced, of the abdomen of the male normal. Ventral genital plate of the female divided into 2 sclerites which bear spines. Dorsal genital plate vaulted and rounded anteriorly. Aedeagus of the male tubular with a large endophallus, connected with the phallobase by a membrane which bears numerous teeth ventrally. Claspers straight, thin, covered with spines and hairs.

Key to the Ethiopian and Madagascar species of Eucampsipoda

Females

1. Only 2 long setae near the posterior margin of the spinose area of the abdomen (rarely none, 3 or 4). 6–8 long setae at the hindmargin of tergite 6 (Fig. 110). **Eucampsipoda africanum** Theodor

— Long setae on dorsum of abdomen more numerous, arranged in 2 groups, one of shorter setae behind tergite 1 + 2 and a group of longer setae posteriorly. 2

2. Anterior group of setae divided into halves of about 12 setae each. Posterior group also divided into halves of 5–7 very long setae in the middle of the dorsum. Tergite 6 elliptical with a row of 8–10 long setae posteriorly with a gap in the middle. Dorsal genital plate longer than wide. Ventral plates narrow, triangular, with a row of 5–6 peg-like spines at the dorsal edge. Anal sclerite short, wider posteriorly, with 2 long setae and several small spines on the surface. Anal segment very short. Middle East (Figs. 106, 107). **Eucampsipoda hyrtlii** Kolenati

— Arrangement of long setae similar, but both groups not divided into halves and there are one or two pairs of long setae between the anterior and the posterior group. Tergite 6 with only 6–8 setae posteriorly. Dorsal genital plate as wide as long. Ventral plates broad, with a row of 6–7 short spines (not pegs). Anal sclerite long and narrow. Anal segment rounded. Madagascar (Fig. 111). **Eucampsipoda madagascarensis** Theodor

Males

1. Peg-like spines on the claspers very small, arranged in a single irregular dorsal row of 7–9 pegs. Aedeagus very long and narrow (0·52 mm.), 13 times as long as wide at the base. Madagascar (Fig. 112). **Eucampsipoda madagascarensis** Theodor

— Pegs on the claspers larger, arranged in 2 rows, aedeagus shorter. 2

2. Pegs all large, arranged in 2 rows from the tip to about the middle of the clasper. Pegs of both rows of about equal size. Parameres small, triangular with a minute hair. Aedeagus long (0·43 mm.), 8 times as long as wide at the base. 2–4 small spines at the apex of sternite 5. Middle East (Figs. 108, 109). **Eucampsipoda hyrtlii** Kolenati

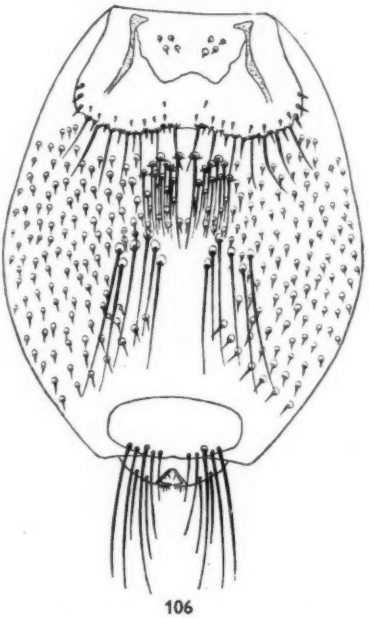
— Lateral dorsal row consisting of 5–8 large pegs, beginning near the tip of the clasper. Inner dorsal row consisting of 7–8 pegs which are very small or spine-like near the tip and larger basally. Parameres large with 3–4 small hairs. Aedeagus shorter (0·38 mm.), about 7 times as long as wide at the base. Only thin hairs at the apex of sternite 5. Ethiopian. **Eucampsipoda africanum** Theodor

1. **Eucampsipoda hyrtlii** Kolenati, 1856

Head and thorax as for the genus.

Abdomen, female (Fig. 106). 2 groups of about 12 thin setae each behind and close to the hindmargin of tergite 1 + 2. 2 groups of 5–7 very long setae in the middle of the dorsum. Tergite 6 elliptical, bare, with 8–10 long setae at the posterior margin. Anal segment very short.

Genital plates (Fig. 107). Ventral plate divided into wing-shaped halves, each of which bears about 6 short, peg-like spines at the inner dorsal corner. Dorsal plate large, rounded anteriorly, longer than broad, vaulted, fused at its posterior edge



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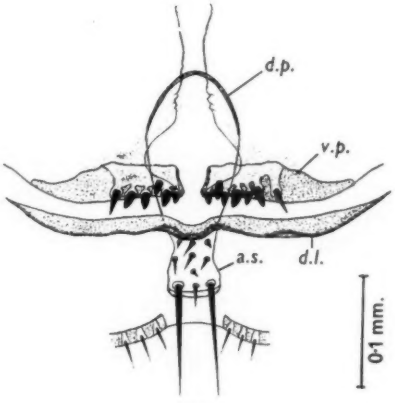

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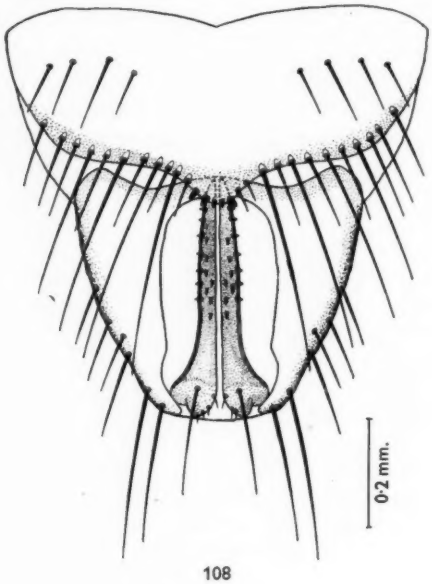
Figs. 106, 107. *Eucampsipoda hyrtlii*. 106, female, abdomen, dorsal. 107, female, genital plates. *a.s.*, anal solente; *d.l.*, dorsal lip; *d.p.*, dorsal plate; *v.p.*, ventral plate.

with the anal sclerite. It forms a lip which covers the genital opening from above. The anal sclerite has a small more strongly sclerotized plate in the middle which bears 2 longer setae at the posterior margin and a few small spines on the surface.

Abdomen, male (Fig. 108). Tergites 3–6 narrow, with marginal rows of moderately long setae. 2–4 long setae in the middle of the rows of tergites 4–6. Sternite 5 triangularly produced in the middle of the posterior margin, with 2 longer and 2 very small spines at the apex. These spines are absent in some specimens.

Genitalia (Fig. 109). Claspers long (0·43 mm.), straight and pointed in dorsal view. In profile, the tip of the clasper is widened and has a short tooth ventrally, close to the tip. There are 2 rows of 6–8 short, thick pegs on the dorsal surface, from near the tip to about the middle of the clasper. The lateral row begins nearer the tip and the dorsal row reaches further basally. A row of thin hairs laterally

near the tip. A long seta dorsally near the base. Basal arc triangular, with a long anterior process. Aedeagus cylindrical with oblique anterior opening, 0·43 mm. long, 8 times as long as wide at the base.



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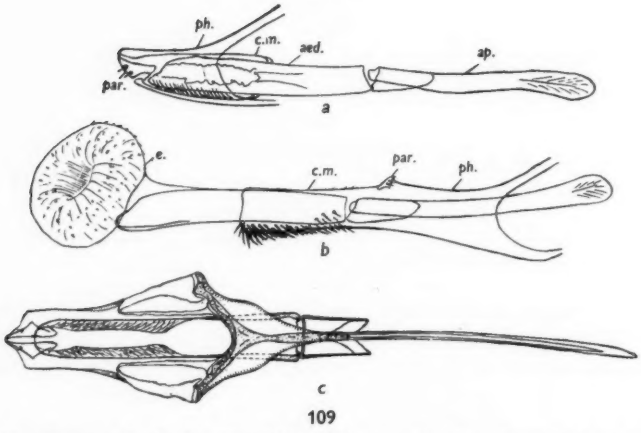

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Figs. 108, 109. *Eucampsipoda hyrtlii*. 108, male, sternite 5 and genital area. 109, male, genitalia: *a*, resting position, profile; *b*, extended position; *c*, resting position, dorsal. For lettering see Theodor & Moscona (1954, p. 172, Figs. 7*a* and 7*b*).

Distribution. *E. hyrtlii* has been identified with certainty only from the Middle East (Arabia, Egypt, Palestine, Syria). It possibly occurs in East Africa where *Rousettus aegyptiacus aegyptiacus* occurs, which is the only species on which *Eucampsipoda hyrtlii* occurs regularly.

2. **Eucampsipoda africanum** Theodor, 1955

This species replaces *E. hyrtlii* all over tropical Africa. The female is easily recognized by the presence of only 2 long setae (rarely none, 3 or 4) at the posterior margin of the spinose area of the dorsum of the abdomen, and by the shape of the genital plates (Fig. 110). The male resembles that of *E. hyrtlii* closely and has to be identified by the characters of the genitalia indicated in the key.

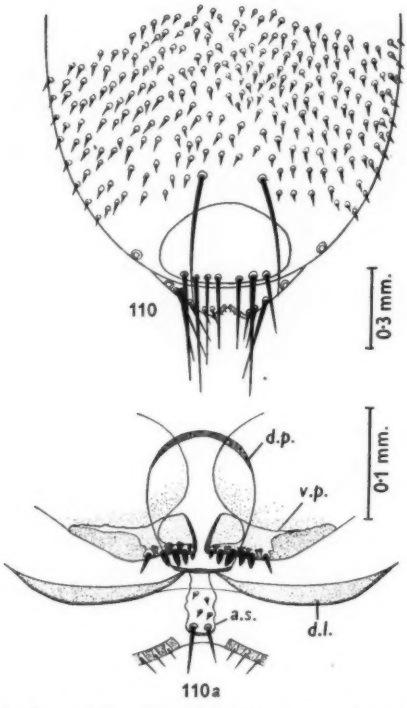


Fig. 110. *Eucampsipoda africanum*, female, abdomen, dorsal and *a*, genital plates; letters as in Fig. 107.

Distribution. West and East Africa, Belgian Congo, South Africa, mainly on *Rousettus aegyptiacus leachi*, but also on *R. aegyptiacus aegyptiacus* and *R. angolensis*. Dr Aellen informs me that 3 specimens from Dakar, Senegal, were found on *R. aeg. aegyptiacus*. Some records of *Eucampsipoda africanum* from *Rousettus aegyptiacus aegyptiacus* will have to be checked as the 2 subspecies of *R. aegyptiacus* have often not been differentiated.

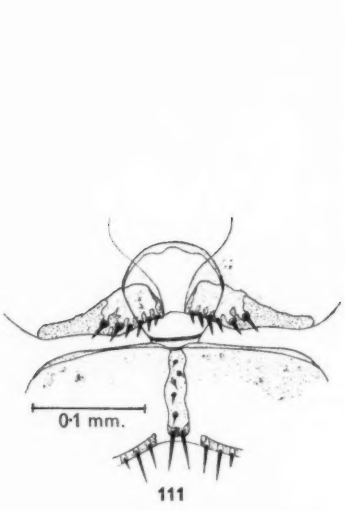
3. **Eucampsipoda madagascarensis** Theodor, 1955

This species also resembles *E. hyrtlii* closely and has to be distinguished from it mainly by the structure of the genitalia as indicated in the key (Figs. 111, 112).

Distribution. Madagascar on *Rousettus madagascarensis*.

Genus **Dipseliopoda** Theodor, 1955

Diagnosis. Head moderately compressed laterally. Eyes with 2 large, well-separated ocelli on a wide pigmented base, protruding from the surface (Fig. 113). Palps of *Eucampsipoda* type. Labella of labium as long as the theca or longer. Thorax as in *Eucampsipoda*. Spines of thoracic and abdominal ctenidia thick and blunt. One larger and 1 or 2 small notopleural setae. Legs also as in *Eucampsipoda*, with 2 rings and short setae in the middle. Abdomen of male as in *Eucampsipoda*.



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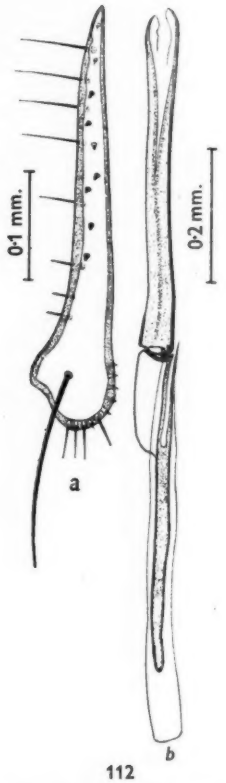

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Figs. 111, 112. *Eucampsipoda madagascarensis*. 111, female, genital plates. 112, male, genitalia: *a*, clasper; *b*, aedeagus.

Sternite 5 with an armature of spines at the posterior margin. Genitalia of *Eucampsipoda* type, with an endophallus which continues in a long tube or without endophallus. Abdomen of female with reduced segmentation. Tergites 1 and 2 fused. Ventral genital plates divided into halves or partly fused. They bear setae or spines and cover the genital opening from below as in *Cyclopodia*.

The 3 species of the genus known so far have been described in a previous paper (Theodor, 1955).

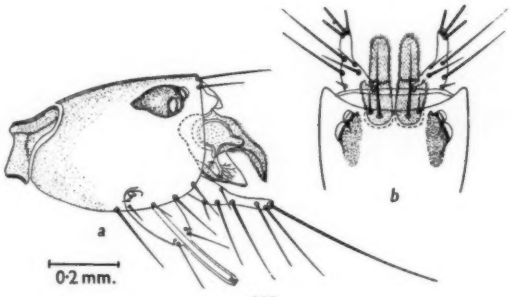
Key to the species of the genus Dipseliopoda

Females

1. Dorsum of the abdomen covered with short setae only. Tergite 6 narrow, with 2 notches at the anterior margin and about 20 setae posteriorly. There is only a single genital plate with about 12 setae at the posterior margin. Anal sclerite large, elliptical, with numerous small hairs on the surface. **Dipseliopoda arcuata** Theodor

— Long setae on the dorsum of the abdomen. 2

2. A group of 10–14 long setae in the middle of the dorsum. Tergite 6 elliptical, with 9–12 setae posteriorly. Genital plate divided into rectangular halves with 5–8 setae posteriorly. Anal sclerite small, elliptical, with 2 longer and some shorter setae posteriorly (Figs. 114, 115). **Dipseliopoda biannulata** Oldroyd



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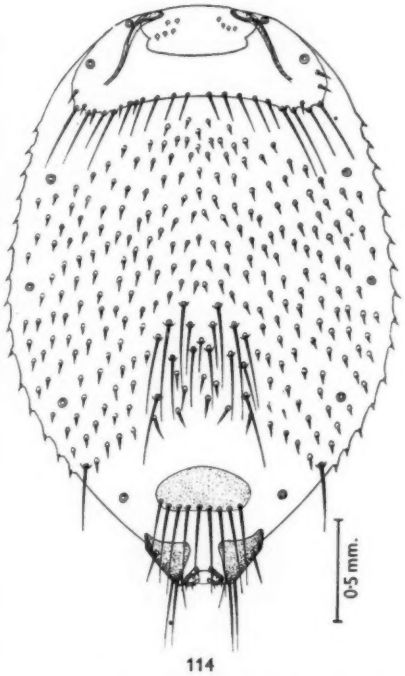

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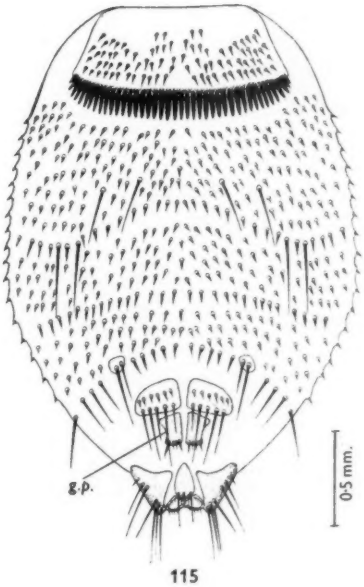

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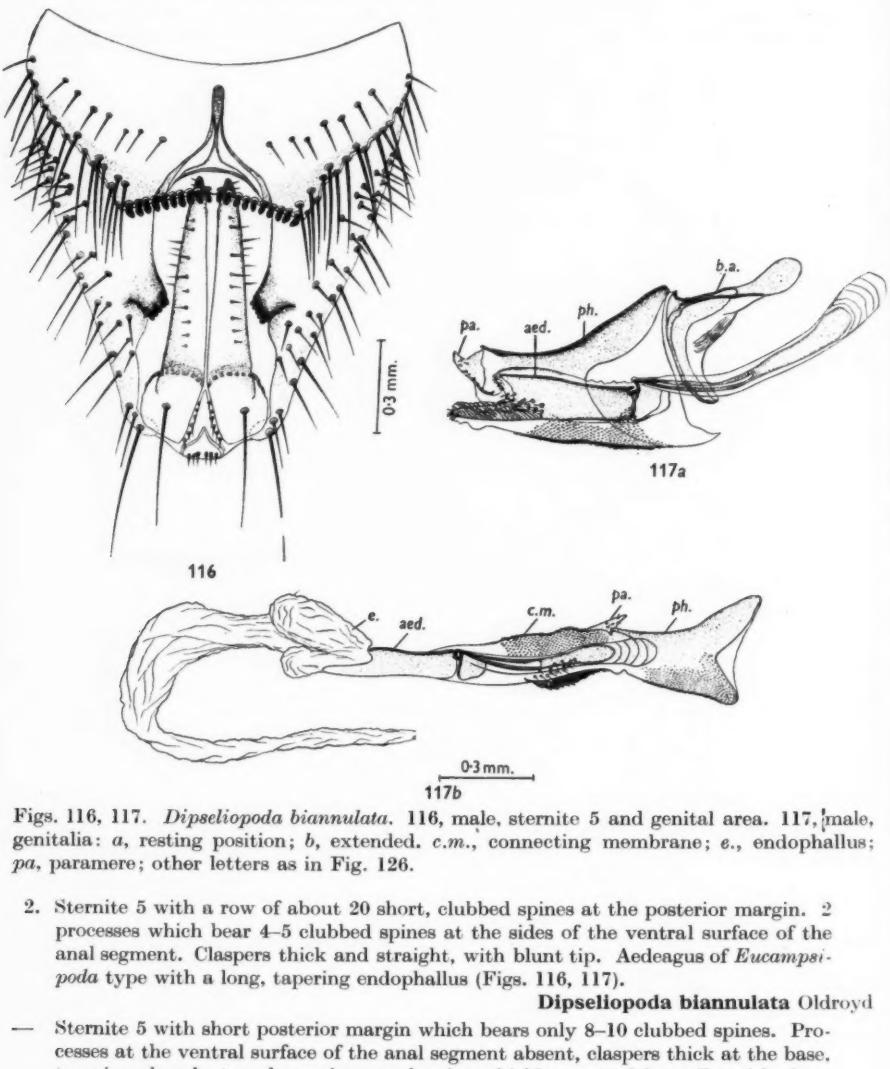
Figs. 113–115. *Dipseliopoda biannulata*. 113, head: *a*, profile; *b*, dorsal. 114, female, abdomen, dorsal. 115, female, abdomen, ventral.

— Dorsum of the abdomen with a dense group of about 300 very long setae. Tergite 6 narrow, without any setae. Ventral genital plate broad, rounded, with long and shorter setae posteriorly (Figs. 118–120). **Dipseliopoda setosa** Theodor

Males

1. Sternite 5 with 2 broad lateral processes posteriorly and a deep concavity between them. A row of about 60 long, curved, dagger-like spines along the concavity (Fig. 123). **Dipseliopoda arcuata** Theodor

— Sternite 5 pentagonal, without lateral posterior processes. 2



Figs. 116, 117. *Dipseliopoda biannulata*. 116, male, sternite 5 and genital area. 117, male, genitalia: *a*, resting position; *b*, extended. *c.m.*, connecting membrane; *e.*, endophallus; *pa*, paramere; other letters as in Fig. 126.

2. Sternite 5 with a row of about 20 short, clubbed spines at the posterior margin. 2 processes which bear 4–5 clubbed spines at the sides of the ventral surface of the anal segment. Claspers thick and straight, with blunt tip. Aedeagus of *Eucampsipoda* type with a long, tapering endophallus (Figs. 116, 117).
Dipseliopoda biannulata Oldroyd

— Sternite 5 with short posterior margin which bears only 8–10 clubbed spines. Processes at the ventral surface of the anal segment absent, claspers thick at the base, tapering sharply to a long pigmented point, thickly covered laterally with short spines. Aedeagus long and narrow, tapering to a point, without endophallus (Figs. 121, 122). **Dipseliopoda setosa** Theodor

1. **Dipseliopoda biannulata** Oldroyd, 1953

Length 3·5–4 mm. Colour dark brown.

Head (Fig. 113). Eyes with double ocelli on a wide pigmented base. The ocelli are large, half spherical and more clearly separated than in the genus *Basilia*, but not as far as in the genus *Cyclopodia*. The surrounding of the eyes is pigmented. Labella of labium slightly shorter than the theca.

Thorax. Mesonotum widening posteriorly. One long and 1 or 2 short notopleural setae. Tibiae long, cylindrical, with 2 rings and moderately long setae in the middle.

Abdomen, female (Figs. 114, 115). Tergite 1 + 2 concave posteriorly, with short setae in the middle of the posterior margin and long setae laterally. Dorsum of abdomen membranous, covered with short setae except in the posterior quarter. A group of 10–14 long setae in the middle near the posterior margin of the spinose area. Tergite 6 elliptical, bare on the surface and with a row of 9–12 long setae at the posterior margin. Anal segment short, conical. Abdominal ctenidium consisting of 40–44 long, blunt, thick teeth. Sternite 6 divided into lateral sclerites with long and short setae posteriorly and a transverse row of short setae on the surface. Ventral genital plate divided into rectangular halves with a row of 5–8 setae posteriorly. Anal sclerite elliptical, with 2 longer and some short setae posteriorly.

Abdomen, male (Fig. 116). Tergites with marginal rows of moderately long setae, bare on the surface. Sternite 5 pentagonal, with a row of about 20 thick, clubbed spines at the posterior margin. Anal segment ventrally with 2 broad processes in the middle of the sides which bear 4–6 spines, similar to those of sternite 5.

Genitalia (Fig. 117). Basal arc triangular, with a long anterior process. Claspers thick and straight, tapering to dark, blunt point. A long seta dorsally near the base and a row of short hairs on the dorsal surface. Phallobase broad, with teeth at the ventral surface. Aedeagus of *Eucampsipoda* type, with a long ventral distal process. Connective membrane with 2 stripes of strong teeth ventrally and some long, thin spines laterally. Endophallus without teeth, with 2 dorsal and 2 ventral lobes, continuing into a long tapering tube. Parameres triangular, with upturned tip.

Distribution. *Nigeria*: Ondo (types). *Cameroons*: Buea and Nyassoso caves, *Rousettus angolensis*, 52 spec. (Eisentraut). *Belgian Congo*: Basa Nfinda caves, Thysville, *Rousettus aegyptiacus*, *R. angolensis*, 30 spec. (Leleup); Mt Wago, Lendu plateau, *Chaerephon faini*, 2 spec. (Fain). *Sudan*: Katire near Torit and Talanga forest, Imatong mountains, *Rhinolophus eloquens* or *R. landeri lobatus*, 7 spec. (Hoogstraal), 4 spec. (Owen); Ruwenzori, 2 spec. (Wollaston). *Kenya*: Kakamega, *Rousettus* sp., 20 spec. (Carcasson).

2. **Dipseliopoda setosa** Theodor, 1955

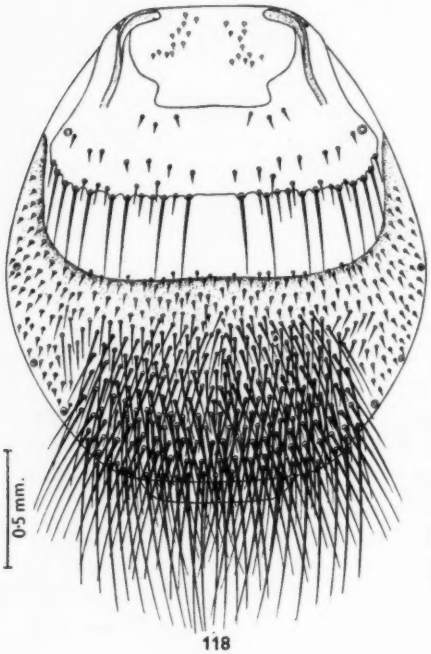
Length 3·5–4·5 mm. Colour dark brown.

Head. Labella of labium 1·5 times as long as the theca. 4 long setae at the anterior dorsal margin. Thorax as in *D. biannulata*.

Abdomen, female (Figs. 118, 119). Tergite 1 + 2 with a row of long setae posteriorly. Some short spines between the long setae towards the middle. Behind the tergite

a bare space as long as the setae of the marginal row of tergite 1 + 2. Dorsum of the abdomen covered with a dense group of about 300 very long setae. Tergite 6 narrow, without setae. Abdominal ctenidium with about 38 thick, blunt teeth. Sternite 6 divided into triangular lateral sclerites with long and short setae posteriorly.

Genital plates (Fig. 120). Ventral plates very wide, broadly rounded and partly fused, covering the genital opening from below. They bear a row of long and short



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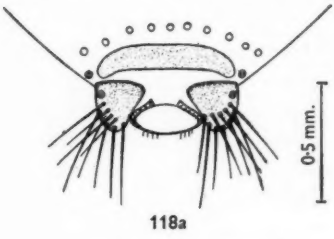

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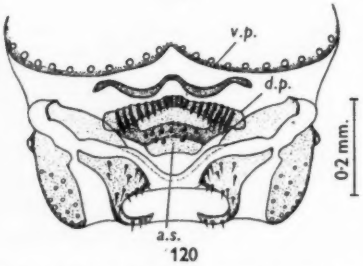

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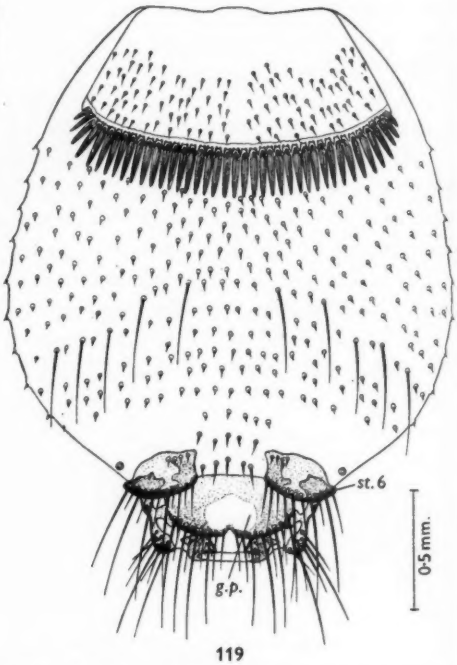

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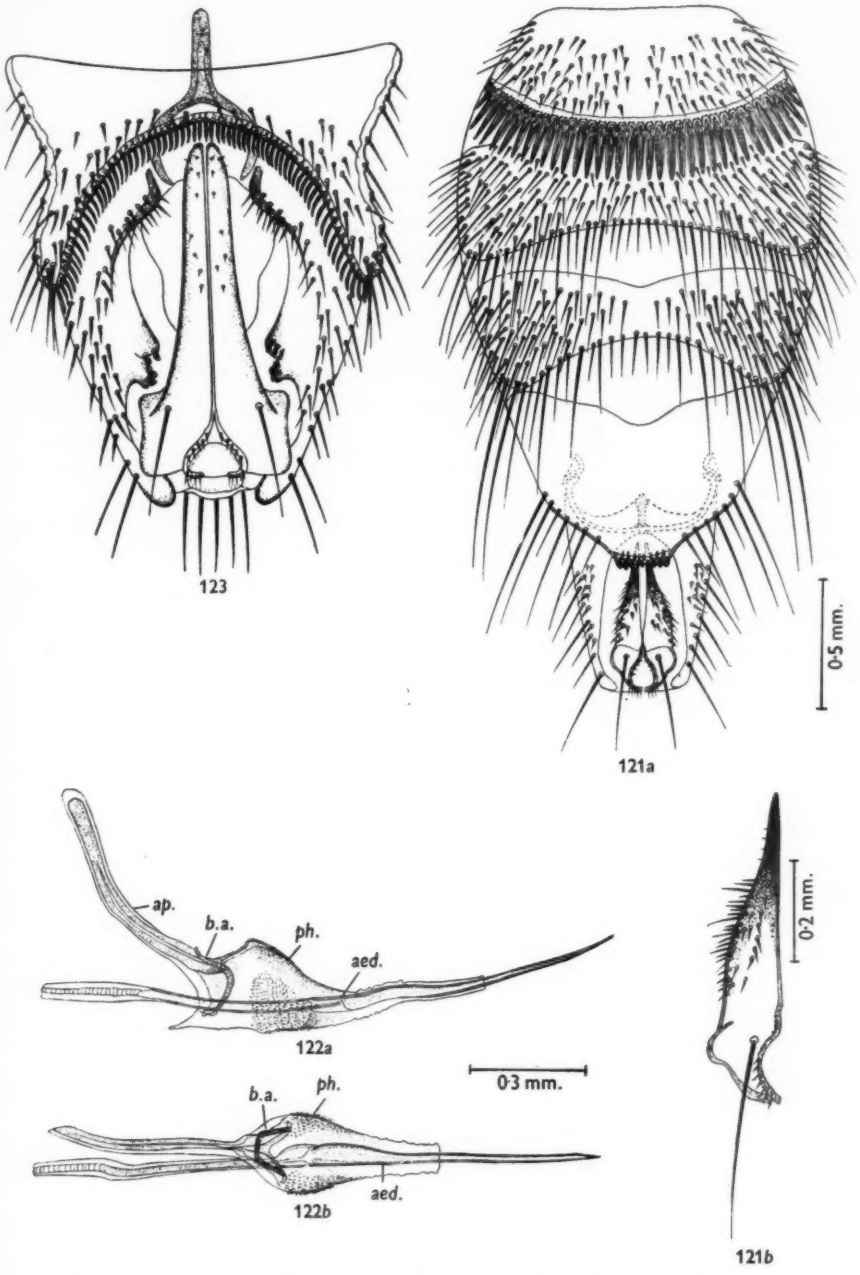
Figs. 118–120. *Dipseliopoda setosa*. 118, female, abdomen, dorsal: *a*, tergite 6 and anal segment. 119, female, abdomen, ventral. 120, female, genital plates; letters as in Fig. 107.

setae posteriorly as on sternite 6. Dorsal plate narrow, strip-like, with a concave posterior margin which bears a row of short spines.

Abdomen, male (Fig. 121). Tergite 1 + 2 triangular, with a marginal row of long and short setae and 3–4 thick spines laterally. Surface covered with setae in the posterior half. Tergites 3–6 narrow, with dense rows of setae posteriorly and short



Figs. 121, 122. *Dipseliopoda setosa*. 121, male, *a*, abdomen, ventral; *b*, clasper. 122, male, genitalia, partly extended: *a*, profile; *b*, dorsal. *ap.*, apodeme; other letters as in Fig. 126.

Fig. 123. *Dipseliopoda arcuata*, male, sternite 5 and genital area.

setae on the surface. Anal segment very narrow, nearly cylindrical, about as long as tergites 3–6 together. Sternite 5 triangularly produced posteriorly with a row of 8–10 thick, clubbed spines at the posterior margin.

Genitalia (Fig. 122). Claspers thick at the base, tapering to a long, darkly pigmented point. A long seta dorsally near the base. The claspers are thickly covered with spines and setae at the sides and on part of the dorsal surface. Phallobase conical, with small teeth ventrally and at the sides, and connected with the aedeagus by a membrane as in *Eucampsipoda* but without spines. Basal arc triangular with a very long apodeme, which is much longer than in any other species of Nycteribiidae examined. Aedeagus very long and narrow, tapering to a fine point. Endophallus absent. The aedeagus is partly fused with a very long apodeme.

Distribution. Kenya: Mt Menengai, *Rousettus lanosus*, 10 spec. (Hoogstraal); Ruiru, *Eidolon helvum*, 2 ♀ (van Someren); Ruwenzori, 1 ♀ (Wollaston). *Tanganyika*: Bagilo, Uluguru mountains, *Rousettus lanosus kempi*, 1 ♀ (Loveridge).

3. **Dipseliopoda arcuata** Theodor, 1955

Length 3–3·5 mm. Colour reddish brown.

Head as in the other species of the genus. Labella of labium two-thirds of the length of the theca.

Abdomen, female. Tergite 1 with a row of moderately long and shorter setae posteriorly. Dorsum of abdomen covered with short setae which become longer posteriorly. There are no long setae. Tergite 6 narrow, tapering laterally, with 2 notches in the anterior margin and with a row of about 20 long setae posteriorly. Abdominal ctenidium with about 55 teeth. Sternite 6 bare with 2 narrow, triangular sclerites which bear a row of setae at the posterior margin. There is only one genital plate with 10–20 setae at the posterior margin, of which 2 are longer than the others. Anal sclerite large, elliptical, with numerous short setae.

Abdomen, male (Fig. 123). Tergites 3–6 narrow, with moderately long setae posteriorly, bare on the surface. Anal segment long, about as long as half the abdomen. Sternite 5 with 2 broad lateral processes and between them a wide concavity at which stands a row of about 65 curved, long, dagger-shaped spines. Some long setae at the tip of the lateral processes. 2 rounded processes with short setae anteriorly and 2 processes with 3–4 short clubbed spines as in *D. biannulata* farther posteriorly at the sides of the anal segment.

Genitalia. Claspers long and straight, with a long seta dorsally near the base and without any other setae except a few minute hairs. Basal arc rounded, with long anterior process. Phallobase and aedeagus similar as in *D. biannulata.* Parameres triangular.

Distribution. Abyssinia: Addis Ababa, 10 spec. (Jannasch & Goertz). *Uganda*: Kampala, *Hypsignathus monstrosus*, 1 ♀ (Hopkins).

Genus **Cyclopodia** Kolenati, 1863

Diagnosis. Head dorsoventrally compressed. Eyes with 2 more or less deeply separated ocelli with pigmented bases. Palps broad, flattened, with short setae along the margins. Dorsal process of basal segment of the antennae broad.

Labella of labium as long as the theca or longer. 4–5 minute spines at the posterior margin of the theca.

Thorax. Mesonotum widened posteriorly. 1–6 notopleural setae. Haltere groove cover present or absent. Ctenidia with thick, blunt spines. Oblique sutures well marked. Coxae 1 very long, club-shaped. Tibiae long, cylindrical, with 3 rings and short setae in the middle.

Abdomen, female. Tergites 1 and 2 fused. Dorsum of abdomen membranous, either without sclerites or tergite 6 present. Postspiracular sclerite without setae. Ventral genital plate with spines posteriorly, covering the genital opening from below or sternite 7 not transformed into genital plate.

Abdomen, male. With normal segmentation. Sternite 5 with or without a row of spines.

Genitalia. Aedeagus membranous, tube-shaped, deeply invaginated inside the abdomen in the resting position. Aedeagus apodeme absent in some species. (Some Oriental species, for which a separate subgenus will have to be created, have differently built genitalia.)

1. **Cyclopodia greeffi** Karsch, 1884

The species has been resdescribed by Falcoz (1924) and the present description is intended to complete his description in some details.

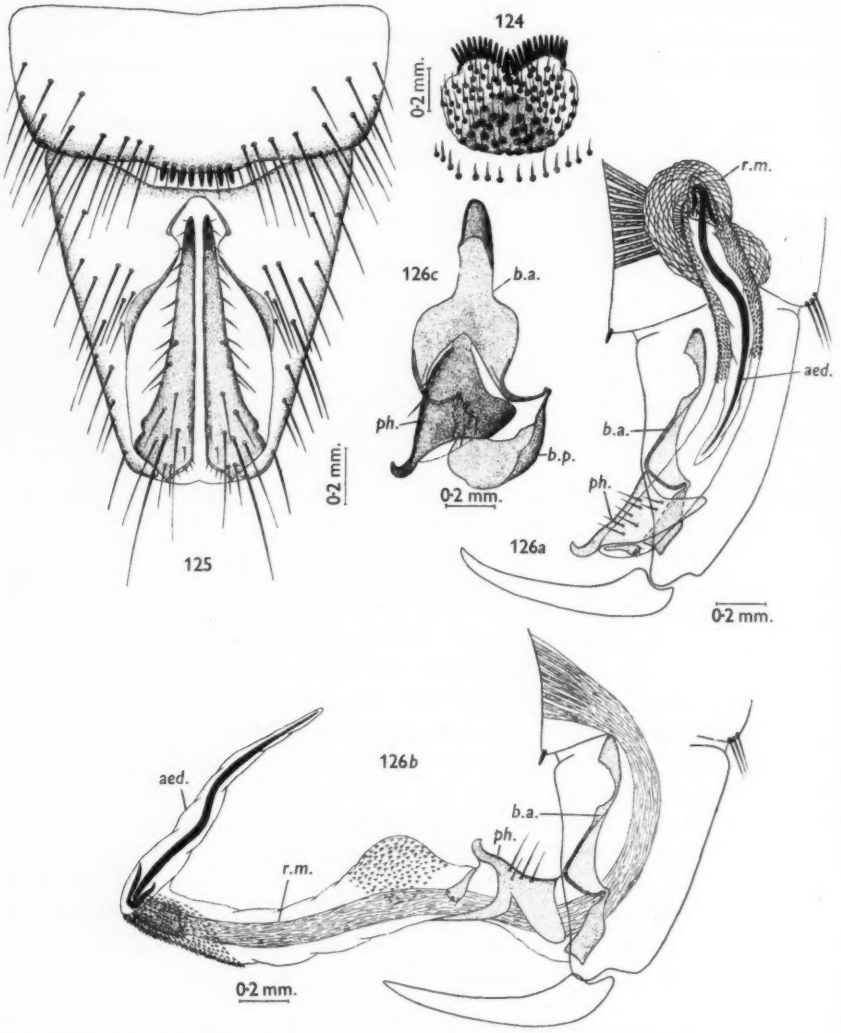
Head bare dorsally, with 2 setae at the anterior margin. Labella of the labium longer than the theca. 2 groups of 5 minute spines at the posterior margin of the theca.

Thorax. Mesonotum moderately widened posteriorly. One notopleural seta. Haltere grooves open.

Abdomen, female. Tergite 1 + 2 large, triangular, with concave posterior margin which bears a row of setae which are longer laterally. Short spines on the surface, particularly in the lateral posterior parts of the surface. Dorsum of the abdomen membranous, covered with short spines which are very short anteriorly and become gradually longer posteriorly and laterally. The last 2 or 3 rows of spines anterior to the long setae are very long and thick. The posterior part of the abdomen is covered with a rectangular group of about 70–80 long setae in 7–8 rows. The posterior row reaches farther laterally than the anterior rows. The dorsum is bare between this group of setae and the anal segment. Anal segment short, conical, with 2 rows of long setae around the anus. Sternite 1 + 2 rectangular, with a ctenidium of 40–44 long, thick teeth. 1–2 rows of short spines along the anterior margin and 2–3 rows of longer spines along the posterior margin before the ctenidium. Ventral surface covered with short spines. The spines of the marginal rows are not much longer than those of the surface. There are some longer setae in the marginal rows of sternite 3 at the sides. The rows of spines on sternite 6 are shorter than on the preceding sternites, with 1–2 rows of short spines on the surface and a marginal row of short setae. The rows of the surface and the marginal row do not reach to the sides of the sternite. Genital plate (Fig. 124) divided medially, with a curved row of long, blunt spines in each half. Anterior to the plate a group

of 80–100 short spines in a roughly rectangular field. These spines stand on sclerotized bases.

Abdomen, male (Fig. 125). Tergite 1 + 2 as in the female, but the posterior margin is convex and the marginal setae are of about equal length. Tergites 3–6 narrow with dense marginal rows of long and short setae. 2–3 rows of short setae on the surface of tergite 3 and 1–2 on tergite 4. Some isolated short setae on tergite 5 and tergite 6 bare. Anal segment long, conical, with moderately long setae on the



Figs. 124–126. *Cyclopodia greeffi*. 124, female, genital plate. 125, male, sternite 5 and genital area. 126, male, genitalia: *a*, resting position; *b*, extended; *c*, basal arc, phallobase and basal plate; *aed.*, aedeagus; *b.a.*, basal arc; *b.p.*, basal plate; *r.m.*, retractor muscle; *ph.*, phallobase.

dorsal surface except in a median stripe. Sternite 5 longer than sternite 4. Its hindmargin is produced in the middle and bears a row of 5–8 short, thick spines.

Genitalia (Fig. 126). Claspers long, thin, slightly curved, tapering to a long, dark point. A long seta dorsally near the base and some short setae and minute hairs on the dorsal surface. Phallobase with a long, curved hook. Parameres with broadly rounded distal end. Aedeagus tubular, membranous with a long pointed rod in its wall which bears 2 short branches basally. The genitalia of *C. greeffi* have been described in detail previously (Theodor, 1954). The following details have to be added. There is a basal arc on which the phallobase articulates as in other genera. The arc has the shape of a broad plate with a long, blunt anterior process and 2 lateral posterior processes which articulate with a basal plate which also exists in other genera. Both basal arc and basal plate form a frame around the basal part of the phallobase. Two lateral processes at the anterior ventral part of the phallobase give support to the connecting tube near the base.

Distribution and material examined

East Africa. *Uganda*: Kampala, *Eidolon helvum*, 24 spec. (Perry); Mbali, 3 spec. (Garnham). *Sudan*: Torit, *E. helvum*, 1 ♀ (Hoogstraal); Lafon, Equatoria province, *E. helvum*, 15 spec. (Owen); Wau, 1 ♂ (Reid). *Tanganyika*: Magrotto near Tanga, *Rousettus angolensis*. *Zanzibar*: 4 spec. (Loveridge); 1 spec. (Anders.).

West Africa. *Senegal*: Faticu, *Eidolon helvum*, 1 ♀ (Wonder). *French West Africa*: Segou, *E. helvum*, 2 ♀ (Rousselot). *Liberia*: Lenga town, *E. helvum*, 5 spec. (Bequaert). *Ivory coast*: Type of *Cyclopodia rubiginosa*, 1 spec. (Alluand). *Togo*: Cap Baudenon, *Eidolon helvum*, 8 spec. *South Nigeria*: Ogbomosho, 20 spec. (Pomeroy); Lagos, *E. helvum*, 48 spec. (Strachan). *Cameroons*: Buea, *E. helvum*, 3 spec. (Eisentraut); Yaounde, 4 spec. (Rageau); Ossidinje, Cross River, 15 spec. (Pomeroy); Kribi, *E. helvum*, 2 spec. (Aellen); Foulassi near Sangmélima, *E. helvum*, 2 spec. (Perret). *French Equatorial Africa*: Gambousa, Bateke plain, 2 spec. (Beatty). *French Central Africa*: Tuburi marshes, 1 ♂ (Talbot); Irabellaban, Baguezane mountains, Aïr, 2 spec. (Chopard). *Fernando Po*: 2 ♂. *Portuguese Guinea*: Gunnal, *Epomophorus* sp., 6 spec. *São Thome Island*: *E. helvum*, 2 spec. (Barns), 23 spec. (Urich).

Belgian Congo. Leopoldville, *E. helvum*, 7 spec. (Bequaert); Leverville, 1 ♀ (Vanderijst); Kivu, Kisenji, 1 ♀ (van Sacceghem); Bukavu, Kivu, *Rousettus* sp., 2 spec. (Grandjean); Boma province, *E. helvum*, 4 spec. (Gerard); Stanleyville, 1 ♀ (Collart); Vista, 20 spec. (Schwetz); Kuleponge, *E. helvum*, 2 spec. (Vrydagh); Lusanga Mwanza, *E. helvum*, 20 spec. (Dartenelle).

2. **Cyclopodia greeffi arabica** Theodor, 1954

This subspecies resembles *C. greeffi* in most respects. In the female the group of long setae on the dorsum of the abdomen consists of only 35–50 setae in 5–6 rows. The group of spines on the genital plate consists of about 65 spines in about 6 rows. In the male the only difference noted is in the shape of the parameres which have a pointed distal end.

Distribution. Ta'izz, Yemen, *Eidolon sabaeum*, 16 spec. (Hoogstraal); Lahej, Aden, *E. sabaeum*, 6 spec. (Yerbury).

3. **Cyclopodia dubia** Westwood, 1835

There is no recent description of this species since Speiser's short description in 1901.

Length 3·5–4 mm. Colour brown.

Head dorsally quite bare, without any setae at the anterior dorsal margin. A row of short setae at the anterior ventral margin with a longer seta posteriorly. Labella of labium markedly longer than the theca. 5 minute spines in each half of the posterior margin of the theca. Eyes with ocelli which are less deeply separated than in *C. greeffi*.

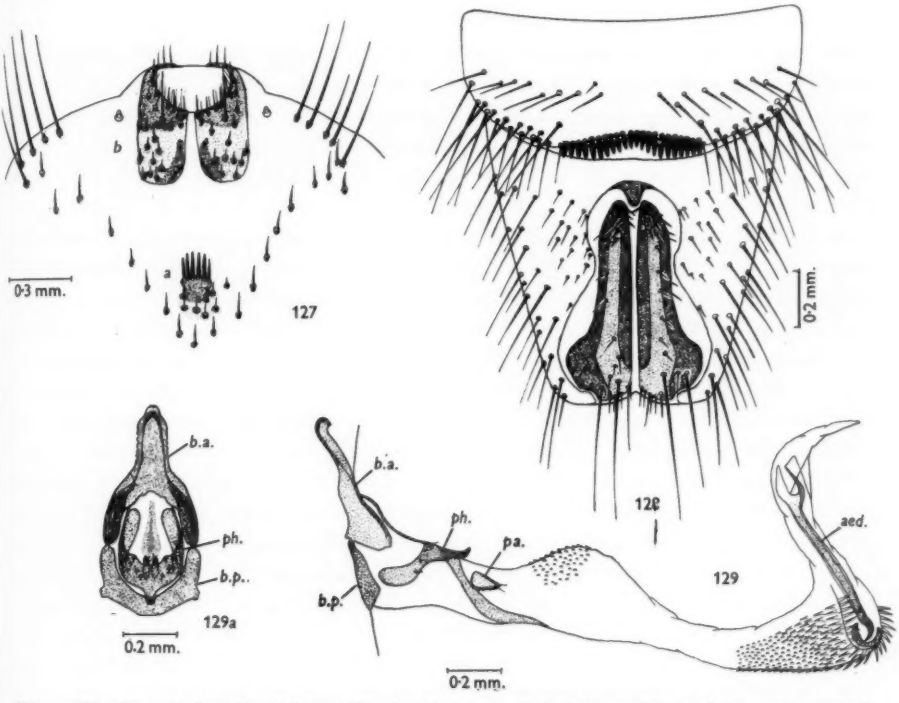
Thorax slightly wider than long, rounded anteriorly. Thoracic ctenidia with 16–18 thick, blunt teeth. Mesonotum rather wide anteriorly, widening posteriorly. One notopleural seta. Median sternal suture narrow, indistinct in the middle. Oblique sutures forming an angle of about 95°. Haltere grooves open. Legs rather short, tibiae with 3 well-marked rings and short setae in the middle.

Abdomen, female. Tergite 1 + 2 triangular, with a marginal row of longer setae laterally and shorter setae in the middle. 3–4 rows of short spines in the posterior part of the surface. Dorsum of abdomen membranous, with minute spines in the middle, longer spines at the sides and on the pleurae and 2–3 rows of still longer spines posteriorly. 2–3 rows of longer setae at the posterior part of the pleurae, between spiracles 5 and 6 and between 6 and 7. A bare space between the posterior row of the dorsum and the anal segment. Anal segment very short, consisting only of a narrow strip on the dorsal surface. These strips widen ventrally into rectangular plates which are covered with short setae. A row of short setae surrounds the anus. Sternite 1 + 2 trapezoid, rather short, with a ctenidium of about 40 thick, blunt teeth, 4–5 rows of short setae in the posterior part of the surface and 1–2 rows along the anterior margin. Ventral surface covered with short spines so that the limits of the sternites are difficult to make out except in distended specimens. There are 12–13 rows of short spines on sternite 3, and 2 long, vertically standing setae in the middle of the posterior row of the surface. Sternites 4 and 5 much shorter, with only 2–3 rows of short spines and 4 long, vertical setae in the posterior row of the surface or in the marginal row. Sternite 6 concave posteriorly with only 2–3 rows of short spines. Genital plate (Fig. 127) small, with 6–10 long spines posteriorly. A few short spines stand close to it anteriorly.

There are two triangular, darkly pigmented spots at the anterior margin of the pleurae, just behind the posterior margin of tergite 1 + 2 and sternite 1 + 2. In cleared preparations under high magnification these spots show a reticulate structure. Nothing like it has been seen in other species of Nycteribiidae.

Abdomen, male (Fig. 128). Tergite 1 + 2 triangular, with 2–3 rows of short spines in the posterior part of the surface. Tergites 1 + 2 to 6 with vertically standing rows of long and shorter setae at the posterior margin. Tergites 3–6 bare on the surface. Tergites 5 and 6 much shorter than the preceding tergites. Anal segment long,

nearly cylindrical, covered dorsally with vertical setae except in a median and an anterior stripe. Some longer setae posteriorly. Sternite 1 + 2 as in the female. Sternites 3 and 4 with marginal rows of long and short setae. There are 4 rows of short spines in the middle and longer setae at the sides of the surface of sternite 3. Sternite 4 similar, but there are only 1–2 rows in the middle and the setae of the premarginal row are longer. Sternite 5 not much longer than 4. A row of 15–20 long, thick spines in the middle of the hindmargin and long setae laterally to them. There is a premarginal row of very long setae with a wide gap in the middle opposite the spines and a row of short setae in the middle of the surface.



Figs. 127–129. *Cyclopodia dubia*. 127, female: *a*, genital plate, and *b*, anal segment, ventral. 128, male, sternite 5 and genital area. 129, male, genitalia, extended: *a*, basal arc, phallobase and basal plate, ventral. *pa.*, paramere; other letters as in Fig. 126.

Genitalia (Fig. 129). Claspers thick and short, straight, with rounded tips which bear a group of strong spines. Some long setae dorsally near the base and a few short setae on the dorsal surface. A rounded process at the anterior ventral margin of the anal segment opposite the tips of the claspers. Basal arc triangular with a long, blunt anterior process and a deep posterior concavity. Basal plate strip-like, curved. Phallobase short, with a deep concavity at the basal margin and a short, thick, apical hook. Several short setae at the base. Parameres triangular, with short hairs at the distal margin when the genitalia are extended. The basal bulb of the tube of the genitalia is wide, with scale-like teeth dorsally and posteriorly.

Connective tube with long spines ventrally near the distal end. Aedeagus membranous, short, with a short, straight rod and a semicircular basal sclerite. The rod is bifid distally and forms a partial frame around the gonopore.

Material examined

Madagascar: Grande forêt de l'Est, *Eidolon dupreanum*, 2 ♀ (Grandidier); Ankarana, Fanihy cave, *Pteropus* sp., 20 spec. (Paulian); Tananariva, *Eidolon dupreanum*, 8 spec. (de la Bethie); Tsimbozaza, 7 spec. (Paulian); Andramborata near Fianarantsoa, 18 spec. (Tipton).

DISTRIBUTION

After the systematic position of the various species has been defined, some information about the distribution of the Ethiopian Nycteribiidae can be given. But, in spite of the extensive material examined, there remain large gaps in our knowledge and it is often not clear whether the absence of records from an area is due to lack of collecting or to non-occurrence in the area. Sufficient material is available only in the case of a few species.

It might be assumed that the distribution of ectoparasites like the Nycteribiidae which pass nearly all their life on the host, except for short periods when the females leave the host to deposit the puparia, would coincide with that of their hosts to a large extent, as they are quite independent of the environment in respect to food, and independent to some extent of fluctuations of temperature and humidity in the rather uniform microclimate in the fur of the bat. It appears, however, that this is not the case and the distribution of the parasites is in many cases much narrower than that of their hosts.

Only 3 species of Nycteribiidae have been found to be distributed widely in Ethiopian Africa. *Nycteribia schmidlii scotti* occurs all over the area, mainly on species of *Miniopterus* and less frequently on species of *Rhinolophus*, *Eptesicus*, *Pipistrellus* and *Hipposideros*. No specimens have been examined from the countries west of Nigeria. The distribution of *Penicillidia fulvida* is similar, according to the material examined, but shows a large gap between 10° and 25° lat. south. It will probably be found in the eastern part of this zone. There is very little material of Nycteribiidae available from the western part of this zone (S.W. Africa, Angola), which is largely desert and dry savannah.

Eucampsipoda africanum seems to have a similarly wide distribution. It has been found in South Africa, Nyasaland, the Belgian Congo, West and East Africa. The species of *Eucampsipoda* are mainly parasites of the genus *Rousettus*. *R. aegyptiacus aegyptiacus* is known from the Middle East, from Cyprus and Syria in the north to Egypt, and a closely allied form, *R. arabicus* is known from Arabia and eastwards to N.W. India. *R. aeg. aegyptiacus* has also been recorded from Angola, from Gabon to the south-east corner of the Cameroons and, as Dr Aellen informs me, from Dakar, Senegal. The species has been recorded from the Belgian Congo, but it is not certain that it has been differentiated from *R. aeg. leachi*, which occurs in South Africa, from Cape Town to Natal along the coast, and extends north to Kenya. A series of specimens from Kenya which were identified originally as

R. aeg. aegyptiacus proved to be *R. aeg. leachi* when they were re-examined by Dr C. C. Sanborn of the Chicago Nat. Hist. Museum.

Eucampsipoda hyrtlii, the specific parasite of *R. aeg. aegyptiacus*, has been identified so far only from the Middle East, including Arabia. All specimens of *Eucampsipoda* from West and East Africa and from the Belgian Congo proved to be *E. africanum*. Some of them are from the south-eastern part of the country, where *R. aeg. aegyptiacus* occurs. *Eucampsipoda africanum* thus seems to replace *E. hyrtlii* in Ethiopian Africa, although *R. aeg. aegyptiacus*, the specific host of *Eucampsipoda hyrtlii*, has a wide distribution in it.

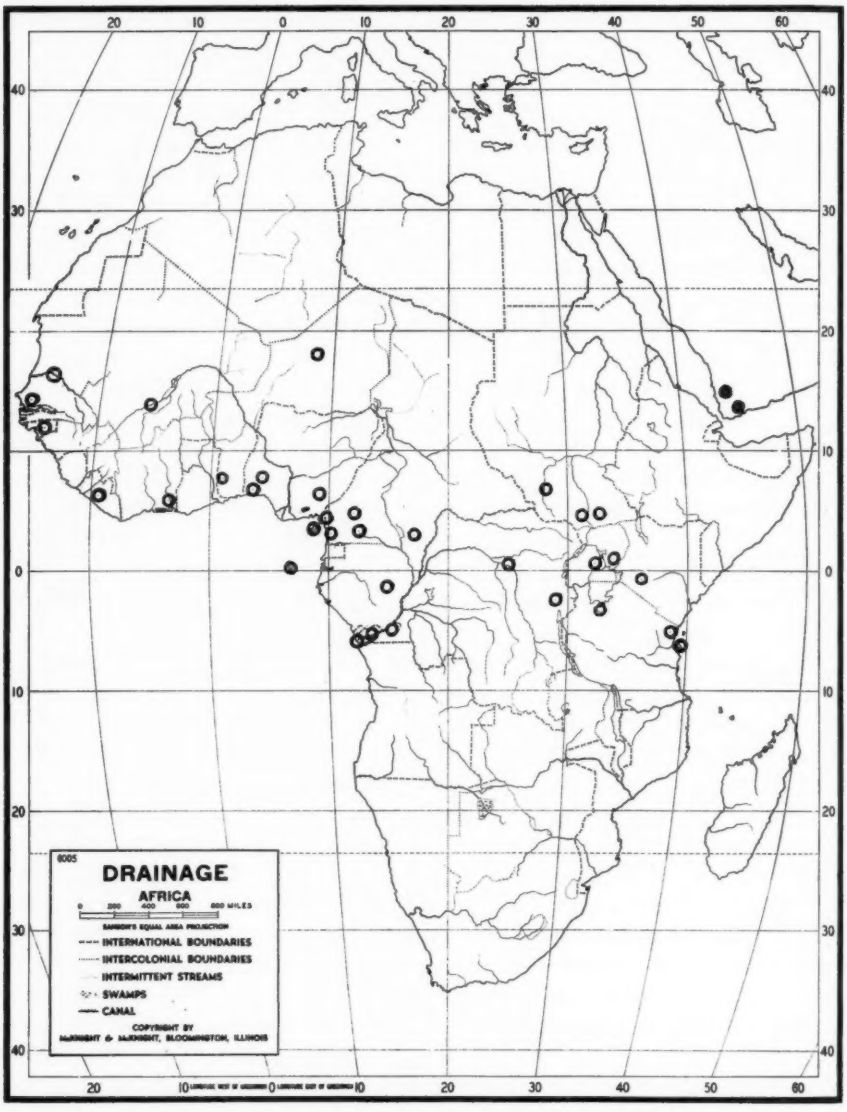
Some species occur mainly in a belt across the continent, between 10° lat. north and south. *Tripselia blainvillii*, *Basilia ansifera*, *Penicillidia pachymela* and *Cyclopodia greeffi* are species of which sufficient material is available which have this type of distribution. *C. greeffi* occurs farther to the north, nearly to 20° lat., in the western part of the zone. Several other species show a similar distribution, but there is too little material to define their distribution exactly (*Basilia bouvieri*, *Dipseliopoda biannulata* (Maps nos. 1–3). Some of these species occur mainly on a single species of bat with a much wider distribution. Thus, *Tripselia blainvillii* is usually found only on *Taphozous mauritianus*, which is distributed all over Ethiopian Africa and the Madagascar Region, including Mauritius. *C. greeffi* is a nearly specific parasite of *Eidolon helvum* which has a similarly wide distribution. *Basilia ansifera* is mainly found on species of *Pipistrellus*, a genus which occurs throughout the whole area. This kind of distribution is difficult to understand as the ecological conditions in the belt vary widely from west to east, from tropical rain-forest to the East African highlands and savannah. More detailed information about the ecology of the bats concerned will be needed before an explanation can be attempted.

A third type of distribution seems to be that of the subgenus *Stylidia*. Its species are found in the eastern part of South Africa and north to Kenya and Uganda, but each species or subspecies has a restricted distribution within this area. There is, however, not enough material of most of these species to define their distribution accurately. Only a few specimens of a South African species, *Nycteribia scissa*, or a closely related form, and a male of *N. inopinata* have been examined from the Cameroons and 2 specimens of *N. ovalis* from Sierra Leone.

The other species seem to be restricted in their distribution as indicated in the descriptions. In some cases, however, the distribution will obviously prove to be much wider. Thus, for example, *N. ovalis* has been found in Sierra Leone, in Durban in South Africa and in Tanganyika. The species will probably be found to be widely distributed throughout Africa.

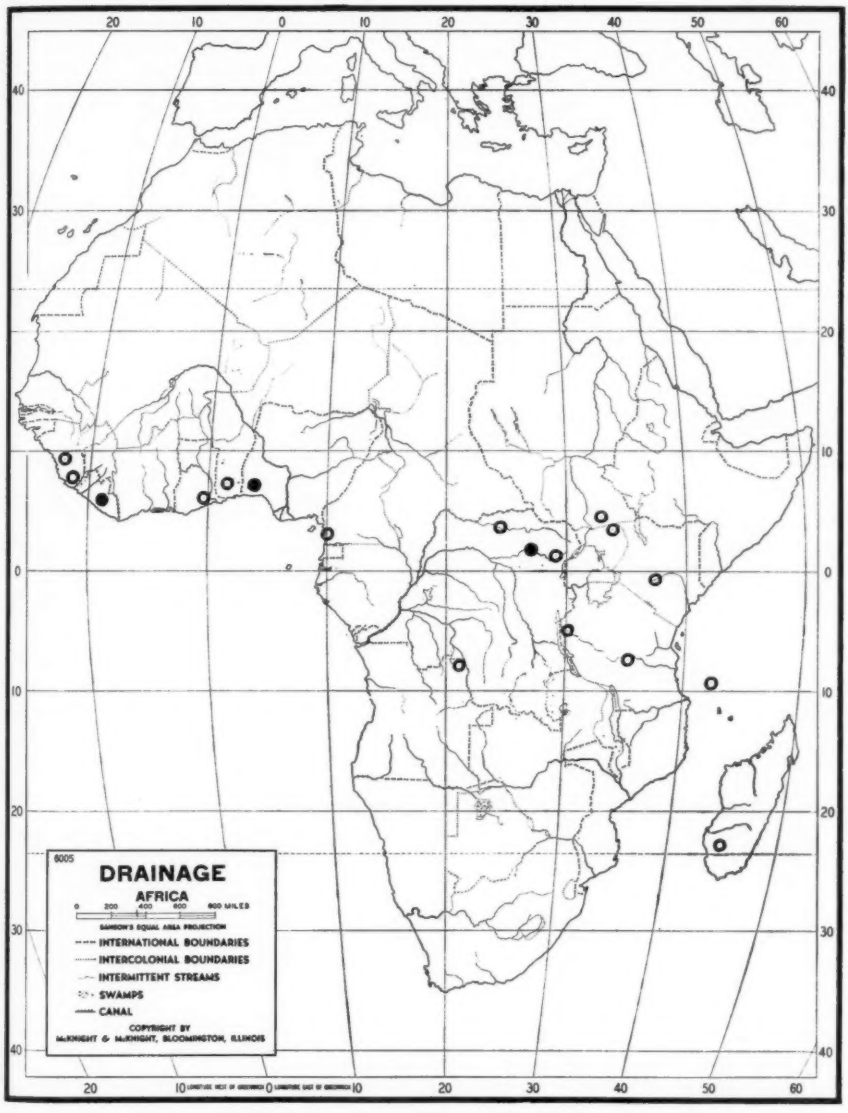
The above are the conclusions which can be drawn from the examination of the material available. The areas of distribution described are obviously incomplete and will probably have to be modified when more material is available. Some of the puzzling features of distribution probably cannot be cleared up by the study of Museum material alone, and a detailed study of the ecology of the bats, their roosting habits, migrations, etc., is probably necessary to explain some of the problems mentioned above.

Only one species of Nycteribiidae, *Tripselia blainvillii*, occurs both on the continent and in Madagascar. The other 6 species known from Madagascar are endemic, although a few species of bats occur in both areas (7 out of 30 species known from the island). The record of *Penicillidia fulvida* from Madagascar by Speiser has been shown to be doubtful and the *Penicillidia* from Madagascar has been described here as a new species. Some of the Madagascar species, e.g. *Nycteribia*



Map no. 1. Distribution of *Cyclopodia greeffi* and *C. greeffi arabica* in Africa. ○, *C. greeffi*; ●, *C. greeffi arabica*.

stylidiopsis and *Basilia madagascarensis*, show more resemblance to the Oriental species than to the Ethiopian species of the same subgenus. *Cyclopodia dubia*, however, resembles *C. greeffi* in the absence of the cover of the haltere grooves. *Penicillidia leptothrinax* resembles the Oriental *P. jenynsii* more closely than the African species of the genus while the new species *P. decipiens* is closely related to *P. fulvida*.

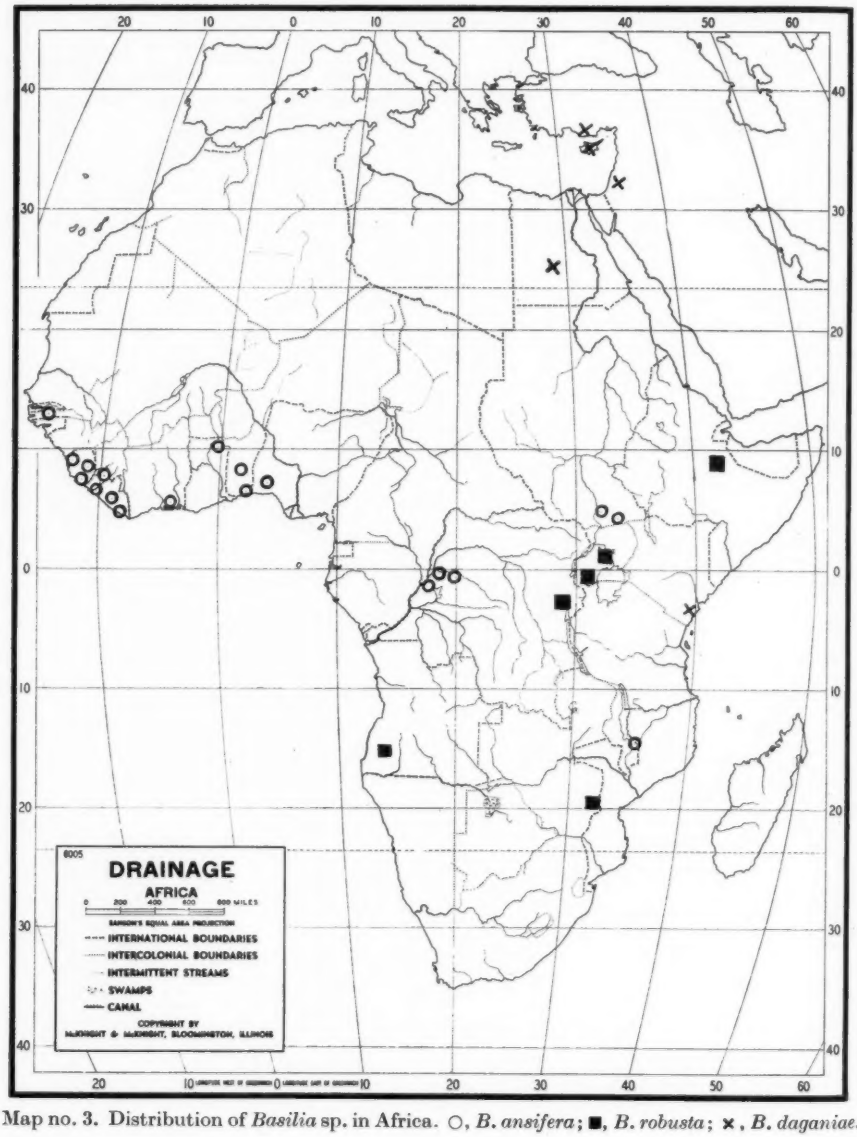


Map no. 2. Distribution of *Tripselia blainvillii* and *T. aequisetosa*.
○, *T. blainvillii*; ●, *T. aequisetosa*.

HOST-PARASITE SPECIFICITY

Here again, the material is sufficient only for a few of the species dealt with. There is no indication of the host in many specimens and some of the older host identifications are probably incorrect.

Some species are specific parasites of a single species of bat, e.g. *Tripselia blainvillii* on *Taphozous mauritianus* and *Cyclopodia greeffi* on *Eidolon helvum*. The



Map no. 3. Distribution of *Basilia* sp. in Africa. ○, *B. ansifera*; ■, *B. robusta*; ×, *B. daganiae*.

differences in distribution of host and parasite have been noted above. *Eucampsipoda africanum* is mainly a parasite of *Rousettus aegyptiacus leachi* and records from *R. aeg. aegyptiacus* in the Congo-Gabon area will have to be checked. Other species occur mainly on a single genus. Thus *Basilia ansifera* and other species of the *B. bathybothyra* group occur mainly on species of *Pipistrellus*.

Nycteribia schmidlii scotti occurs mainly on species of *Miniopterus* (22 lots) and more rarely on species of *Rhinolophus* (6 lots). *Nycteribia scissa* and the other species of the subgenus *Stylidia* occur also mainly on these two genera of bats.

Penicillidia fulvida seems to be quite unspecific. Specimens from 14 different species of bats, belonging to 7 genera and 5 families were examined. These include a record from *Eidolon helvum* which has to be considered as an exception. There is less material of *Penicillidia pachymela* and the records are from *Hipposideros caffer*, *Nycteris* sp. and *Rhinolophus eloquens*, all 3 species belonging to different families.

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THE *IXODES SCHILLINGSI* GROUP: TICKS OF AFRICA AND MADAGASCAR, PARASITIC ON PRIMATES, WITH DESCRIPTIONS OF TWO NEW SPECIES (IXODOIDEA, IXODIDAE)

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(With 37 Figures in the Text)

INTRODUCTION

During the course of a revision of the genus *Ixodes* in Africa one of the most interesting groups investigated was that of the ticks parasitic on primates. Undoubtedly much more collecting from the African primates would be particularly rewarding and presents a virgin field for biological investigation. Up to the present time the only *Ixodes* tick known to infest monkeys in Africa was *I. schillingsi* Neumann, 1901, and even this species is restricted to the *Colobus* monkeys of East Africa.

The present work was initiated when the writer was in receipt of a Leverhulme Research Award and continued in East Africa under a Colonial Welfare and Development grant from the Colonial Office. To these organizations the writer is deeply indebted for financial assistance. The writer also wishes to thank the following for their assistance: the British Museum (Natural History) through the courtesy of Dr G. Owen Evans and Mr E. Browning; Mr Harry Hoogstraal, NAMRU-3, Cairo; Dr Gertrud Theiler, Onderstepoort Research Station, South Africa; Dr E. Dartevelle, Musée Royal du Congo Belge, Tervueren, Belgium and Mr A. J. Wiley, Veterinary Laboratories, Kabete, Kenya, as well as the authorities of the East African Veterinary Research Organization for facilities at their Muguga laboratories and the hospitality of their hostel.

Ixodes schillingsi Neumann, 1901 (Figs. 1–19)

I. schillingsi Neumann (1901), pp. 288–9, female, described from 12 females from *Colobus caudatus* from German East Africa from the Schillings collection (Berlin Museum). Neumann (1910), pp. 170–2, described the male from a specimen found in copula in the province of Gorongoza, Mozambique, G. Vasse *leg.*, 1907 (Paris Museum). Nuttall, Warburton, Cooper & Robinson (1911) based their description of the female and nymph (N. 1171) on 1 female and 2 nymphs from among Neumann's types; their description of the male is condensed from Neumann who stated that the 2 females collected with the male were larger than the types. Rageau (1953) collected specimens of alleged *schillingsi* from the Cameroons but this is a mis-identification.

Material examined. Total 15 females, 6 males, 55 nymphs, 62 larvae. The data

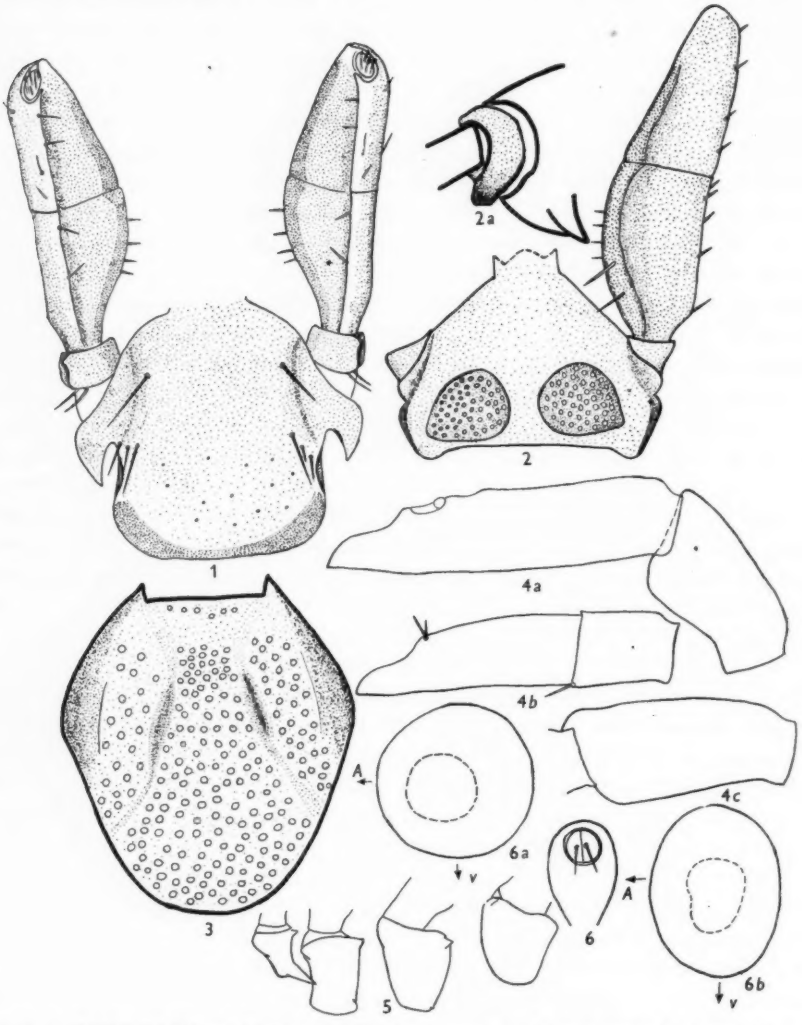
presented here are according to the legends in the vials. 1 female, *Colobus* sp. (HH 3835), Rift Valley Province, Njoro, Kenya, 5. vi. 48, H. Hoogstraal *leg.*; 3 females, *Colobus* sp. (HH 3835–6), Rift Valley Province, Njoro, Kenya, 5. vi. 48, H. Hoogstraal *leg.*; 1 female, *Colobus* sp., Lotti Forest, Equatoria Province, Sudan, –iii. 1950, H. Hoogstraal *leg.* (Hoogstraal collection); 4 males, 7 nymphs, *Colobus* sp. (HH 11197), Arusha, Tanganyika, –vii. 1956, H. Hoogstraal *leg.*; 2 females, 1 male, *Colobus* sp., 1913, Africa (G. H. F. Nuttall collection 2379); 2 nymphs, *Colobus caudatus* 'German East Africa'—'from among Neumann's types', British Museum (Natural History); 2 females, Nairobi, Kenya, T. J. Anderson *leg.*, British Museum (Natural History); 1 female, male monkeys, Cedarwood, Kenya; 1 female, *Colobus* monkey, Nairobi, British East Africa, T. J. Anderson *leg.*, British Museum (Natural History); 3 females, monkey, Cedarwood, Kenya 1929, British Museum (Natural History); 1 female, *Colobus*, Nyeri (A. Wiley collection H 730); the following immature stages are from the Onderstepoort collection: 329, 1 nymph, 1 larva; 347, 17 larvae; 427, 1 nymph, 1 larva; 429, 1 nymph; 444, 1 nymph (all collected in Zanzibar between 13 July and 7 August, 1955); 582, 1 nymph, 8 larvae; 814, 1 larva; 596, 1 nymph, 8 larvae; 561, 1 nymph, 3 larvae; 563, 4 nymphs, 1 larva; 592, 2 larvae; 586, 2 nymphs; 556, 4 nymphs, 1 larva; 552, 3 larvae; 585, 2 nymphs; 598, 1 nymph; 550, 8 nymphs, 3 larvae; 549, 7 nymphs, 7 larvae (all from Pemba Island between 12 and 31 August, 195?); 1 nymph, 1 larva, Cedarwood, Kenya (G. H. F. Nuttall collection 3683); 1 female, *Lophiomys ibeanus* subsp. (crested rat) immature (in captivity), between pads of hind foot, Nanyuki, North Nyeri, Kenya, August 1956, purchased by H. Hoogstraal from R. Hook.

Redescription of female

Length of engorged specimen from tips of palpi to posterior margin 5–7 mm., breadth across front of spiracles 3–5 mm., relatively short when compared with its breadth, narrowed anteriorly, dilated at about mid-length, rounded posteriorly, broad oval body in engorged specimens, spiracles nearer to posterior border than to coxae IV; opisthosoma covered with short white hairs. Colour, basis capituli and scutum dark brown, palps and legs pale brown.

Capitulum (Figs. 1, 2, 2*a*). Length from dorsal ridge to apex of hypostome 0·84 mm., greatest breadth of 0·49 mm. just behind insertion of palpal article 1; basis capituli, a moderately broad equilateral triangle, with lateral margins markedly divergent anteriorly and posterior margin between cornua salient, straight or rarely slightly concave; cornua (if present) broader basally than long and rounded apically; surface smooth or a little rugose in parts, shining. Porose areas large, subtriangular or piriform, broadened transversely, depressed, porose interval narrow and in some specimens evident as a longitudinal depression. Palpi long, more than three times as long as wide, rarely as little as 2·6 times as long as wide, scarcely extending beyond the hypostome, widely separated at their base and bending inwards, lateral margin a mild gradual arc varying slightly in degree of concavity but rarely deeply concave, latero-basal angle swollen; inner margin slightly convex, particularly at about two-thirds the distance along article 2,

thereafter along article 3 almost straight to the apex, which is more sharply curved mesially than laterally; meso-dorsal edge of article 2 and most of article 3 forms a flange (Fig. 2); length of article 2, 0·33 mm., of article 3, 0·33 mm. or in some



Figs. 1–6. *Ixodes schillingsi* female: 1, capitulum, ventral; 2, basis capituli, dorsal; 2*a*, side view of distal portion of basis capituli; 3, scutum; 4, tarsus and metatarsus I (*a*), IV (*b*), metatarsus IV (*c*); 5, coxae I–IV; 6, anal groove; 6*a*, spiracular plate; 6*b*, male, spiracular plate.

specimens article 3 slightly shorter than article 2. Hairs, small and inconspicuous except for two larger ones meso-dorsally and basally. In ventral view, basis capituli slightly broader (when measured across auriculae) than long, posterior margin almost straight, salient, postero-laterally rounded and more heavily

pigmented, converging at about one-third of the length anteriorly to form a slight constriction before widening to the auricular bases; auriculae as large sharp retrograde hooks (Fig. 1); surface of basis capituli generally flattened, but slightly convex near the hypostomal base; auricular surface convex; a pair of moderately long hairs at one-third distance of base of hypostome and origin of auriculae, second pair of similar hairs mesial to and behind origin of auriculae, two shorter hairs latero-ventral to the second pair. Hypostome, length 0·40 mm., relatively broad, lanceolate, greatest breadth basally; denticles long, rounded peripherally, arranged from apex to base as 5 rows of 5/5 files, 2 rows of 4/4 files, 4 rows of 3/3 files, 4 rows of 2/2 files.

Scutum (Fig. 3). Variable in size and shape, length 1·37–1·51 mm., breadth 1·09–1·19 mm., the usual shape observed in the material examined is depicted in Fig. 3, but some specimens compare favourably with Fig. 234 of Nuttall *et al.* (1911); longer than wide, widest at or in front of the middle, producing a shouldered effect, behind greatest width sides rectilinear or slightly sinuous, scapulae short, pointed with moderate emargination. Lateral carinae indicated in some specimens as slight elevations, lacking in others, sharp slope antero-laterally. Cervical grooves indistinct anteriorly where they form a very shallow, wide depression, widening and deepening about mid-length before shallowing out to the postero-lateral margins. Punctations very marked, deep, moderate size, numerous and uniformly distributed except anteriorly between the cervical grooves. Hairs short, strong, white. Surface, in general, bright and shining.

Legs. Moderate length. Tarsi (Figs. 4*a–b*) long, especially those of pairs I and IV, tapering by a short hump fairly abruptly near their extremities, length of tarsus I, 0·7 mm., metatarsus I, 0·38 mm.; tarsus IV, 0·64 mm., metatarsus IV, 0·45 mm.; coxae (Fig. 5) mildly convex, I–III with syncoxal areas, posterior edges trenchant, surface smooth with few hairs postero-externally; coxa I with long, pointed postero-internal spur, postero-internal margins of II and III produced into small but distinct marginal saliences, coxa I with short, pointed external spur, similar spurs on II and III, but these may be much reduced in some specimens, external spur on coxa IV forms a flat triangular projection. Trochanters with slight tooth ventrally at posterior distal extremity of each.

Spiracular plate. Subcircular, diameter 0·3 mm., placed nearer to anus than to coxae IV, macula antero-ventral, moderate number of goblets.

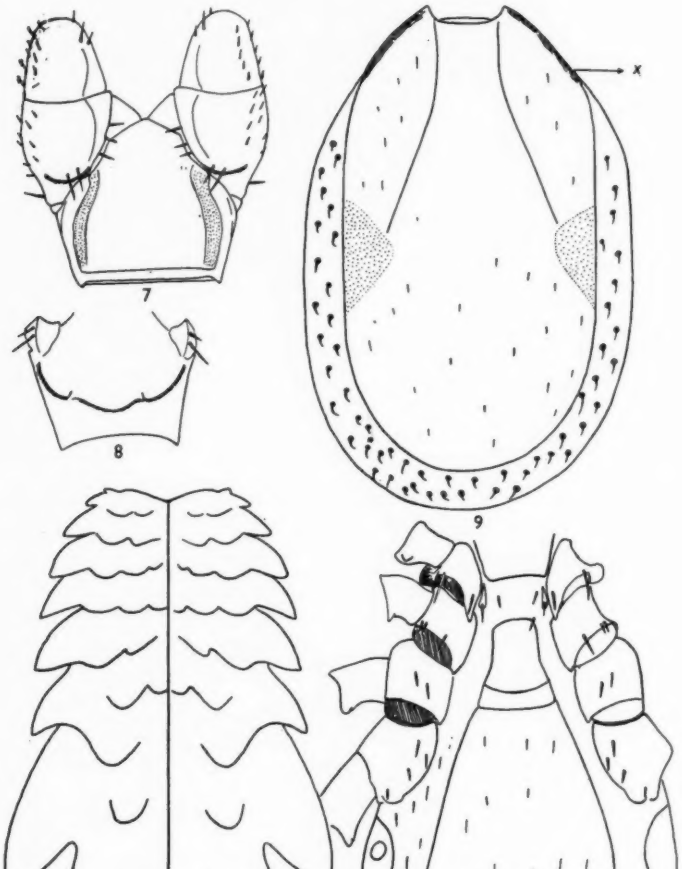
Anal aperture and grooves. Located far back; anal groove forming an ellipse, incomplete posteriorly (Fig. 6). According to Nuttall *et al.* (1911), the ellipse terminates in a short prolongation. I have not observed this in the specimens examined.

Genital aperture. Between coxae IV, genital apron unilobed.

Redescription of male

Body shape elongate oval (Figs. 9, 11), length 2·43 mm., greatest breadth 1·42 mm.; sclerotized parts brown with wide, pale marginal fold; upper margin of spiracle just visible from above.

Capitulum (Figs. 7, 8). Length from dorsal ridge to tip of hypostome 0·5 mm., breadth across dorsal ridge 0·30 mm., posterior margin straight and salient, cornua very small, triangular, lateral borders diverge slightly anteriorly to palpal



Figs. 7–11. *Ixodes schillingsi* male: 7, capitulum, dorsal; 8, basis capituli, ventral; 9, opisthosoma, dorsal; 10, hypostome; 11, opisthosoma, ventral.

insertion thence curve in strongly to hypostomal base; basis capituli slightly broader than long, surface mildly convex between two longitudinal depressions (stippled in Fig. 7) which extend back almost to the dorsal ridge; beyond the

depressions surface elevated and rounded, smooth, shining, reticulately patterned. Colour, periphery black, posterior margin edged by a narrow bar of pale brown pigment, anterior to which is a broader black bar joining up with those of the margins. Palpi short, broad, almost globular, distal margin of article 2 distinctly broader than base of article 3, length about three times greatest breadth; lateral profile of article 2 gently curved, that of article 3 straight, supplied with two or three longitudinal rows of short, white hairs which disappear apically (Fig. 7); mesial profile of articles 2 and 3 convex and with a pronounced dorsal bulge which terminates rather sharply mesially and towards the base of article 2; hairs fewer and longer mesially than laterally and limited to the inner distal margin of article 3, and the proximal half of article 2 where there are two long hairs on the meso-dorsal surface and three smaller hairs on the mesial border; length of article 2, 0·18 mm., length of article 3, 0·16 mm., breadth across distal extremity of article 2, 0·18 mm. Ventrally the rectilinear sides of basis capituli converge to a slightly concave or more rarely straight posterior margin (Fig. 8), transverse ridge strongly developed with a steep slope to the hypostomal base, and declivitous behind; auriculae with a short straight median edge and a long curved oblique outer edge which terminates near the base of article 1, median lobe long across the base and broadly convex behind, the bases of the auriculae and median lobe heavily pigmented, apices paler; ventral edges of palps with strong white hairs, shorter hairs laterally. Palpal article 1 broader ventrally than dorsally, drawn out into a keel-like projection from the outer side of which two moderately strong white hairs arise; mesial faces concave, with a greater overlap dorsally; hairs on meso-ventral edge of palpi longer and stronger than those laterally. Hypostome (Fig. 10) long (0·19 mm.) but distinctly broad, apex flattened and indented in the mid-line, seven strong curved lateral teeth in front of a very large, downwardly projecting basal tooth, the sub-basal tooth of moderate size and curved outline, five distinct rows of crenulations (in files of 3/3 and $3\frac{1}{2}/3\frac{1}{2}$) distally, median crenulations between the fifth and sixth rows without corresponding lateral teeth, sixth row with a single median crenulation, a single crenulation internal to the basal tooth, a large irregular median tooth mesial of the sub-basal lateral tooth and of about the same size.

Scutum. Length 1·80 mm., breadth 0·98 mm. (i.e. somewhat smaller than indicated by Neumann), elongate oval, sides straight and about equally rounded in front and behind, in some specimens the curvature posteriorly is more strongly emphasized than figured (Fig. 9), surface shining, smooth, except antero-laterally where it is wrinkled as in the alloscutum (*X* in Fig. 9), mildly convex between the cervical grooves but slopes appreciably to the periphery; scapulae short, broad, pointed, emargination moderate. Lateral carinae lacking. Cervical grooves shallow, extend back to nearly mid-length and cease in front of a lateral, triangular depression (stippled in Fig. 9). Punctations, moderately large, unequal, absent from three small areas in the posterior third. Hairs short, white, scattered. Marginal fold wide, creamy white in alcohol-preserved specimens, hairs strong, curved, longer than those on the scutum, encircled by pigmented placques.

Ventral plates (Fig. 11). Pregenital plate extends from anterior edges of coxae

III to those of coxae II, sides strongly convergent behind, before becoming parallel anteriorly, rounded distal edge; median plate pentagonal, widest towards posterior end; anal plate racket-shaped; adanal plates longer than broad, front margins straight, inner margins sinuous, outer margins convex, posterior margin straight. Punctations large, more widely separated than those on the scutum: hairs short and scattered, about equal in length to the hairs on the epimeral plates.

Legs. Pale brown, robust, pair IV very long, the last two articles extending beyond the posterior margin of the body. Coxae I–III syncoxae (Fig. 11), posterior margins trenchant; coxa I triangular with long, distinct, pointed spur postero-internally, a slight tuberosity externally; coxae II and III very broad with reduced, but nevertheless distinct spurs internally; coxa IV longer than broad, anterior face concave, and fits up closely against posterior edge of coxa III, coxa IV bears a rounded projection externally; all coxae very close together. Small trochanter spurs on legs I–IV (Fig. 11). Tarsi humped, tarsus I tapering slightly to Haller's organ, with distinct preapical hump before narrowing to the tip, length of tarsus I, 0·65 mm., metatarsus I, 0·33 mm.

Spiracular plate. Large, oval, long axis parallel to that of the body. Macula antero-ventral.

Redescription of nymph

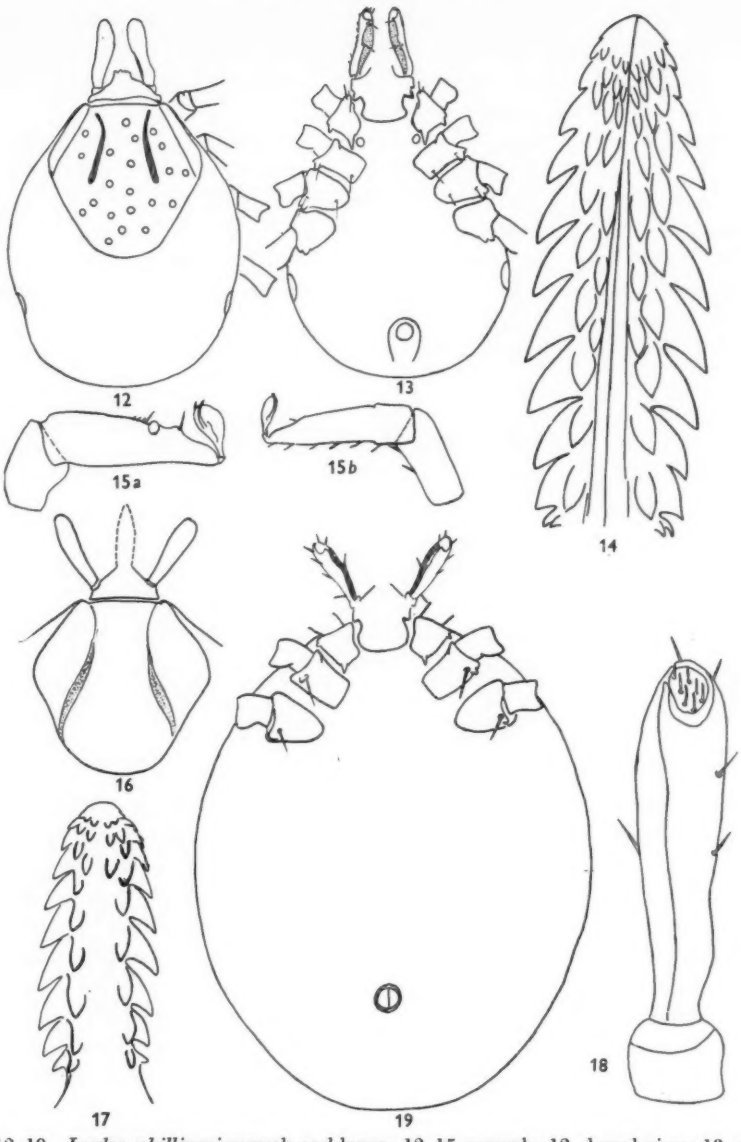
Capitulum (Figs. 12, 13). Overall length 0·31 mm., width of basis capituli across dorsal ridge 0·3 mm., basis capituli more than twice as broad as long, surface smooth, shining, impunctate, posterior margin sinuous with prominent postero-lateral projections. Cornua, if present, weak. Palpi long, narrow, slightly concave laterally with pronounced hump basally, mesially convex; widest about two-thirds palpal length, no distinct suture between articles 2 and 3, combined length of articles 2 and 3, 0·28 mm., hairs few and fine laterally, longer on meso-ventral surface. Ventrally basis capituli constricted about mid-length, posterior margin gently curved, pointed auriculae directed postero-laterally. Surface impunctate. Mesial surface of palp flat. Hypostome (Fig. 14), sides a little convex, apex rounded; denticles arranged from apex to base as an apical 'corona', 3 rows 4/4 teeth, 4 rows 3/3 teeth, 4 rows 2/2 teeth; teeth smaller to mid-line, length about 0·18 mm.

Scutum (Fig. 12). Length 0·53 mm., width 0·53 mm., widest in front of middle, lateral carinae absent; cervical grooves at first convergent, then divergent, short, deep, wide depressions diverging to postero-lateral borders, but not reaching them; surface smooth, shining, reticulate with few large punctations most pronounced between cervical grooves and posteriorly.

Tarsi. As in Figures (Figs. 15*a*, *b*). Coxae: external spurs progressively shorter from coxa II to IV; coxa I with strong internal spur, external spur about half as long (Fig. 13).

Spiracular plate. Located well back from coxae IV.

Anal groove. Converging strongly behind.



Figs. 12–19. *Ixodes schillingsi* nymph and larva: 12–15, nymph: 12, dorsal view; 13, ventral view; 14, hypostome; 15, tarsus and metatarsus I (*a*), IV (*b*). 16–19, larva: 16, capitulum and scutum; 17, hypostome; 18, palp, ventral view; 19, ventral view of entire gorged larva.

Description of larva

Capitulum (Figs. 16, 17). Basis capituli just over twice as broad as long; posterior border straight, drawn out postero-laterally, no cornua. Palpi long (Fig. 18), narrowing proximally and widening distally, length 0·144 mm. Ventrally

basis capituli constricted about midway along, auriculae as mild lateral extensions, not pointed. Surface impunctate. Hypostome (Fig. 17), length 0·13 mm., dentition succeeds apical 'corona' as 3 rows 3/3 teeth, 6 rows 2/2 teeth to the base, lateral and median teeth about equal, pointed.

Scutum (Fig. 16). Length 0·33 mm., width 0·355 mm., greatest width at or slightly in front of mid-length, lateral carinae indistinct, cervical grooves wide, shallow, extending to postero-lateral margins; scapulae very short and rounded, emargination slight. Punctations few, coarse.

Tarsi. Very similar to those of nymphs. Coxae. Coxa I with internal and external spur, inner spur stronger than external spur, coxa II with external spur, coxa III without spurs (Fig. 19).

Related species and remarks. See under *I. rageaui* n.sp.

Ixodes rageaui n.sp. (Figs. 20–33)

I. schillingsi (Rageau, 1953, 399–411), see remarks below.

Holotype. Female from *Lasiopyga cephus*, Yaoundé, Cameroons, deposited at the Onderstepoort Research Station.

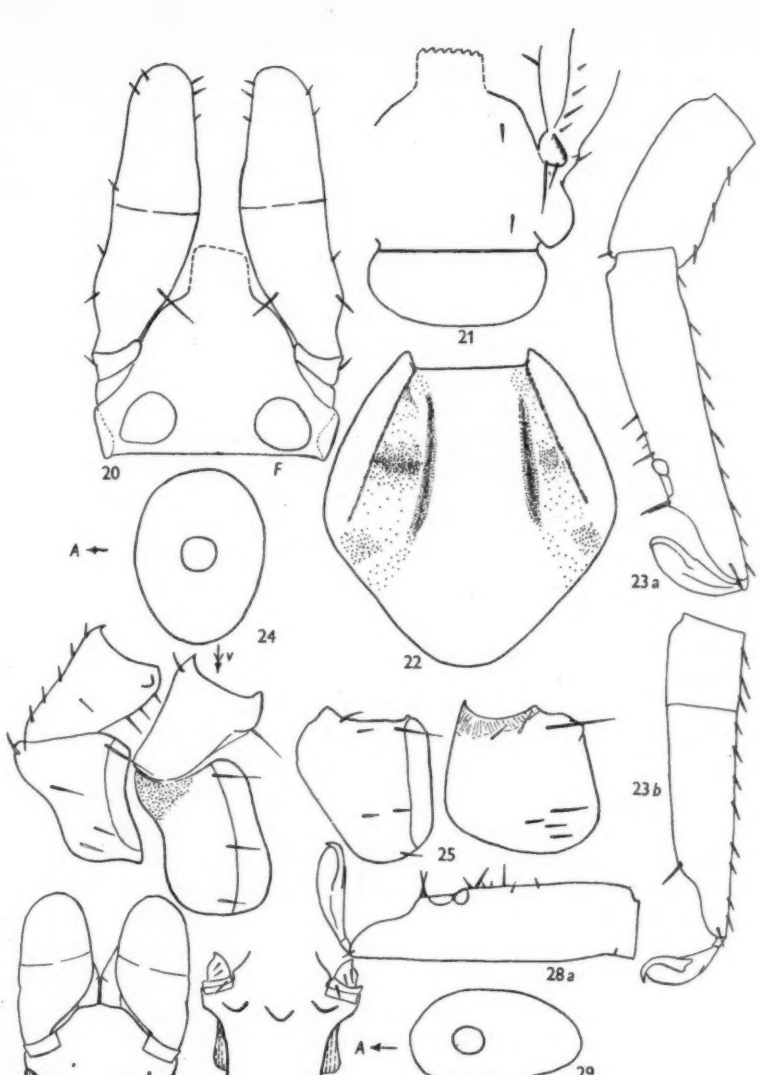
Allotype. Male from *Lasiopyga cephus*, Yaoundé, Cameroons, deposited at the Onderstepoort Research Station.

Paratypes. Male and female, with same data as holotype and allotype. Deposited in the Musée Royal du Congo Belge, Tervueren.

Description of female

In alcohol-preserved specimens scutum and margin of basis capituli dark brown, legs and palps straw-coloured; alloscutum reddish brown. Spiracles much closer to last pair of coxae than to the anus. Opisthosoma bears short fine hairs.

Capitulum (Figs. 20, 21). Length from basis capituli to rostral base 0·34–0·35 mm., breadth across dorsal ridge 0·46 mm.; surface generally flat except for a convexity antero-laterally between article I and hypostomal base, posterior margin straight, lateral margins divergent, elevated and rounded (limits indicated by dotted line in Fig. 20). Porose areas small, rounded to subtriangular, interporose interval far greater than length of areas. Surface smooth, dark brown with more heavily pigmented margin. Palpi moderately broad; about 3·5 times as long as broad, laterally and basally article 2 distinctly bulged, becoming slightly concave anteriorly, mesially palps convex, particularly along proximal two-thirds, length of article 2, 0·32 mm., maximum breadth of article 2, 0·21 mm., length of article 3, 0·27 mm., hairs few and short. Ventrally, basis capituli strongly constricted behind the auriculae, posterior and postero-lateral margins strongly rounded; auriculae as broad and flat plates, directed latero-ventrally (Fig. 21), one pair of hairs at base of hypostome, a comparable pair mesial to auriculae; article 1 with a ventral triangular spur, with two unequal hairs arising from ridge, hypostome missing in both females.



Figs. 20–29. *Ixodes rageaui* n.sp. 20–25, female; 26–29, male. 20, capitulum, dorsal; 21, capitulum, ventral; 22, scutum; 23, tarsus and metatarsus I (*a*), tarsus IV (*b*); 24, spiracular plate; 25, coxae I–IV; 26, capitulum, dorsal; 27, basis capituli, ventral; 28, tarsus I (*a*), IV (*b*); 29, spiracular plate.

Scutum (Fig. 22). Length 1·18 mm., breadth 1·08 mm., widest at or about the middle, roughly diamond-shaped, postero-lateral margins taper more rapidly than antero-lateral margins, posterior extremity narrowly rounded, scapulae drawn out to a point, emargination moderate. Cervical grooves shallow behind scapulae leading to deep depression extending back beyond half scutal length, becoming wider, flatter and shallower to the postero-lateral margins; lateral carinae distinct, straight, extend to level of widest point of scutum but do not reach border, beyond carinae surface slopes gradually; between the cervical grooves surface flattened, beyond the grooves surface undulate (in Fig. 22 the more heavily stippled regions indicate depressions, unstippled areas elevations). Punctations larger and deeper in posterior region of scutum, unevenly spaced; fewer and shallower beyond the cervical grooves and also between grooves anteriorly. Hairs (?).

Legs. Moderate size and thickness, coxae I–III syncoxae (Fig. 25), coxa I with very short broad spur, internal spurs lacking on coxae II and III, small external spurs on coxae IV, coxae IV very broad, hairs of moderate length. Tarsi (Figs. 23*a*, *b*) long, narrowing from proximal to the distal end, slight hump immediately in front of Haller's organ, length of tarsus I, 0·7 mm., metatarsus I, 0·38 mm., length of tarsus IV, 0·65 mm., metatarsus IV, 0·51 mm. Trochanter spurs present on legs 1–4.

Spiracular plate (Fig. 24). Transversely elongate oval, greatest length 0·35 mm., greatest breadth 0·17 mm., macula almost central.

Anal groove. Racquet-shaped as in *I. schillingsi*.

Description of male

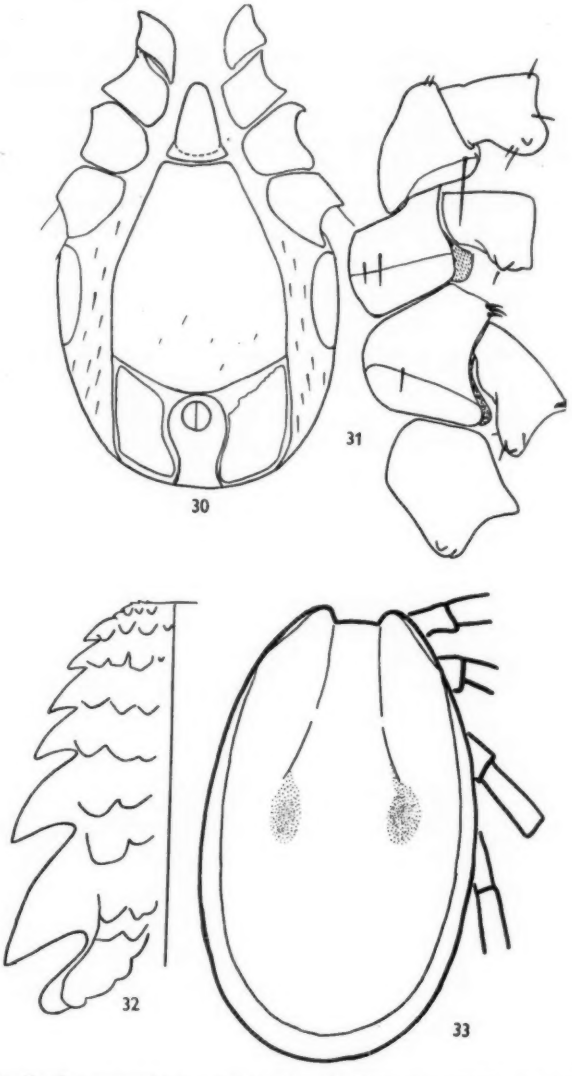
Small, sclerotized parts brown, legs pale yellow in alcohol-preserved specimens.

Capitulum (Figs. 26, 27). Overall length 0·46 mm., breadth across dorsal ridge 0·26 mm. Basis capituli about as long as broad, more or less rectangular, lateral margins sinuous behind the palpal insertion, postero-lateral angles rounded, no cornua; posterior margin straight, postero-lateral angles of the dorsum extend laterally as flanges which are visible from below (indicated by broken lines in Fig. 27); surface generally flattened, pores irregularly distributed. Palpi short, broad, lateral profile of article 2 somewhat undulate, mesial profile as an arc of a circle, apex rounded; length of article 2, 0·16 mm., article 3, 0·15 mm., breadth across junction of articles 2 and 3, 0·14 mm. Ventrally basis capituli long, narrow, sides gently concave, postero-lateral angles rounded, posterior margin almost straight; transverse ridge indicated by paired lobed auriculae and median unpaired lobe, all of approximately same size. Hypostome (Fig. 32), length 0·174 mm., broad, apex flattened, lateral teeth extending almost to the tip. Twelve lateral teeth including the large basal and smaller sub-basal components, crenulations as 5/5 for distal quarter of hypostome, followed by 4/4 to 3/3 with a number of irregular crenulations associated with the basal and sub-basal teeth.

Scutum (Fig. 33). Length 1·77 mm., breadth 0·95 mm., surface convex, more accentuated posteriorly, margins broadly rounded, lateral carinae lacking, cervical grooves shallow, wide, leading to a subcircular depression at about

mid-length. Punctations large, closely set. Scapulae broad, rounded, emargination moderate. Marginal fold narrow, broadest posteriorly, supplied with very short thick hairs, few in number.

Ventral plates (Fig. 30). Pregenital plate triangular, narrowed markedly anteriorly; median plate broad, hexagonal, margins of posterior half almost parallel; anal plate much as in *I. schillingsi*; adanal plates longer than broad, sclerotization in dried specimen, irregular.



Figs. 30–33. *Ixodes rageaui* n.sp. male: 30, opisthosoma, ventral; 31, coxae I–IV; 32, left half of hypostome; 33, opisthosoma, dorsal.

Legs. Moderate length, coxae I–III syncoxae, latter being more extensive than in *I. schillingsi* (cf. Figs. 11, 31), internal spur of coxa I short, broad, spurs lacking on coxae II and III, slight external spur on coxa IV (Fig. 31). Tarsi shorter and broader than in female (Figs. 28*a*, *b*, cf. 23*a*, *b*), tarsus I with medium hump in front of Haller's organ, length of tarsus I, 0·62 mm., metatarsus I, 0·29 mm., length of tarsus IV, 0·55 mm., metatarsus IV, 0·37 mm.

Spiracular plate (Fig. 29). Large, length 0·35 mm., breadth 0·2 mm., elongate oval, more broadly rounded anteriorly, narrowing posteriorly.

Diagnosis. Like *I. schillingsi* it would appear that *I. rageaui* is primarily a primate-parasitizing tick in West Africa. Separation of these two species can be achieved on the characters of the basis capituli and coxae, amongst others.

Females. I. schillingsi: retrograde, pointed auriculae; porose areas large, interporose distance narrow. Coxa I with long, pointed internal spur, external spurs on all coxae.

I. rageaui: Flat almost rectangular auriculae; porose areas small, interporose distance greater than the diameter of the porose areas. Coxa I with short, broad postero-internal salience, external spurs lacking on coxae II and III.

Males. I. schillingsi: lateral denticles of hypostome not extending to indented apex, basis capituli with small triangular cornua, lateral margins divergent to palpal base, ventral lobe and auriculae broad, coxa I with long, pointed internal spur.

I. rageaui: lateral denticles of hypostome extending nearly to flat apex, basis capituli lacking cornua, lateral margins sinuous to palpal base, ventral lobe and auriculae narrow, coxa I with short, broad postero-internal salience.

Remarks. Rageau (1953) reported these specimens as *I. schillingsi* and in his key to the identification of *Ixodes* ticks from the Cameroons he stated that coxa I has a long internal spine. The forms of the female specimens of *I. rageaui*, which have same host, locality and date information, from the collections of the Musée Royal du Congo Belge and the Onderstepoort Veterinary Research Department agree very well between themselves, and it seems reasonable to believe that they represent the specific form of the material obtained from *Lasiopyga cephus* from the Cameroons. I name the species for Dr J. Rageau because he has added much to our zoological knowledge of the Cameroons.

Ixodes lemuris n.sp. (Figs. 34–37)

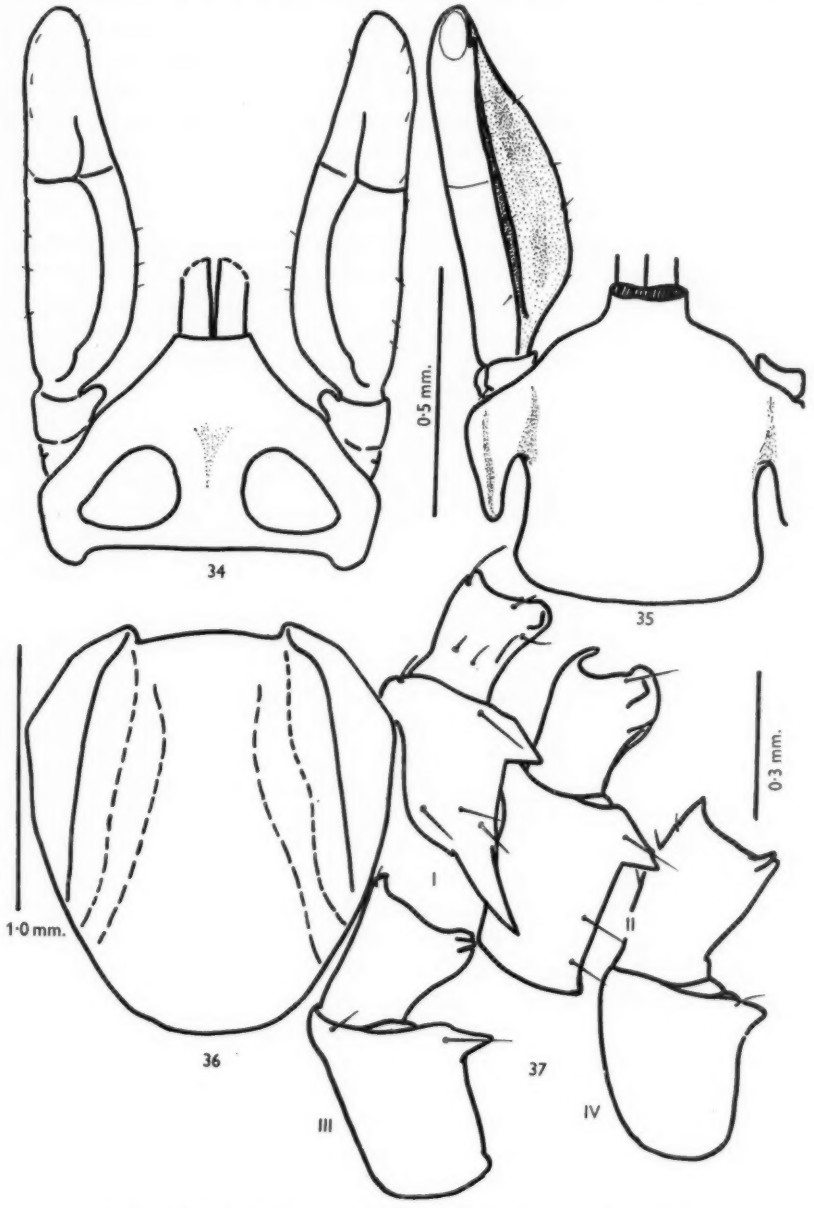
Known only from the holotype female: collected from *Lemur rufifrons*, Ankafana Forest, East Betsileo, Madagascar, Rev. W. Dean Corran *leg.* Deposited in British Museum (Natural History), 1892, 3. i. 2.

Description of female

Sclerotized parts dark brown to black, shiny, alloscutum with moderately long hairs, distinct marginal fold.

Capitulum (Figs. 34, 35). Basis capituli broader than long, breadth 0·69 mm., length from dorsal ridge to hypostomal base 0·52 mm., posterior margin straight, cornua broadly rounded, shorter than their greatest breadth, lateral margins

divergent to palpal insertions. Porose areas large, slightly depressed, piriform and separated by a distance equal to their greatest breadth; slight depression in the interporose interval. Palpi of moderate length, article 2, 0·42 mm., article 3,



Figs. 34–37. *Ixodes lemuris* n.sp. female: 34, capitulum, dorsal; 35, capitulum, ventral; 36, scutum; 37, coxae I–IV.

0·35 mm.; greatest breadth midway along article 2, 0·22 mm., tapering proximally and distally, article 3 tapers mesially and apically in a sharply angled profile, gently convex laterally, lateral profile of both articles straight, except for slight basal indentation, meso-dorsal edge forms a broad flange, article 1 with strong spur meso-dorsally, hairs short. Ventrally basis capituli broad, flat except for gradual slope near the hypostomal base; posterior margin almost straight, postero-lateral angles sharp, slight constriction just behind auriculae; latter large, tapering spur-like projections directed backwards and downwards (not retrograde), auricular surface slightly undulate. Palpal article 1 with flap-like projection ventrally. Hypostome missing.

Scutum (Fig. 36). Length 1·55 mm., greatest breadth of 1·35 mm. in front of the middle. Colour uniformly brown except antero-laterally and anteriorly where it is straw-coloured. Scapulae short, rounded apically, emargination moderate; lateral carinae a pronounced broad ridge curving outwards anteriorly and extending back in a straight line almost to the postero-lateral border, lateral to the carinae a sharp, strong, wide slope, mesially the slope to the carina from the cervical grooves is shorter; cervical grooves as broad valleys, shallow in front and slightly convergent, then divergent back to the postero-lateral border (Fig. 36). Surface between cervical grooves flat and at a lower level than the carinae. Punctations small, moderately deep and uniformly distributed. Glabrous in this specimen.

Legs (Fig. 37). Distal segments of all legs missing in this specimen. Coxae large, generally flat, reticulate patterning with few moderately long white hairs, coxa I with long, broad internal spur which considerably overlaps coxa II; external spur pointed, short and broad; large external spurs on coxae II–IV, internal spurs on coxae II and III reduced to marginal saliences. Strong distal pointed spurs on trochanters I–IV.

Spiracular plate. Closer to coxae IV than to anus, round, macula antero-ventral, with large number of goblets of moderate size.

Anal grooves. Almost completely circular but do not meet posteriorly.

Related species. This species from a lemur in Madagascar bears an undoubted affinity with *I. schillingsi* of Mozambique (Neumann, 1901), Kenya and the Anglo-Egyptian Sudan (Hoogstraal, 1953), but it differs therefrom in having article 2 of the palps very much longer than article 3 (as 1·5:1·0), a prominent meso-dorsal spur on article 1, pronounced ridged lateral carinae on the scutum, larger and deeper punctations on the scutum, cervical grooves as broad valleys extending postero-laterally, the spiracular plate nearer to the fourth pair of coxae than to the anus, and in the shape of the auriculae (cf. Figs. 1, 35). Hoogstraal (1953) has stressed that while the bulk of known endemic Malagasy tick fauna shows close relationship to Oriental fauna, the Malagasy species show marked difference from the Oriental species while those species in the Malagasy fauna that are closely related to the African fauna differ but slightly from their African representatives. The slight differences between *I. schillingsi* and *I. rageaui* n.sp. of Africa and *I. lemuris* n.sp. of Madagascar are additional evidence in support of this statement.

The host relationships of these three species of ticks are also of some interest. Many species and groups of species within the genus *Ixodes* show marked host

predilection. In our present knowledge, we find that *I. schillingsi* occurs on the *Colobus* monkeys of East Africa, *I. rageaui* on *Cercopithecus* monkeys of West Africa and *I. lemuris* on lemurs of Madagascar.

SUMMARY

1. This paper reviews the morphology, distribution and hosts of *I. schillingsi* Neumann, 1901.

2. Two new species, *I. rageaui* from the Cameroons, and *I. lemuris* from Madagascar are described and figured. The latter shows obvious African affinities and is but slightly different from its African relative.

3. *I. schillingsi* is, with the exception of *Lophiomys ibeanus* (crested rat), known only from *Colobus* monkeys, *I. rageaui* from monkeys of the *Cercopithecus* group and *I. lemuris* from *Lemur rufifrons*.

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